ACTA AGRONOMICA
ACADEMIAE SCIENTIARUM HUNGARICAE

TOMUS XXV FASCICULI 1–2

AKADÉMIAI KIADÓ, BUDAPEST
1976

ACTA AGRON. HUNG.

ACTA AGRONOMICA

A MAGYAR TUDOMÁNYOS AKADÉMIA
AGRÁRTUDOMÁNYI KÖZLEMÉNYEI

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SZERKESZTŐSÉG ÉS KIADÓHIVATAL: 1054 BUDAPEST, ALKOTMÁNY UTCA 21.

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Acta Agronomica
2462 Martonvásár, Postafiók 19.

Ugyanerre a címre küldendő minden szerkesztőségi és kiadóhivatali levelezés.

Megrendelhető a belföld számára az Akadémiai Kiadónál (1363 Budapest Pf 24. Bankszámla 215-11488), a külföld számára pedig a „Kultúra" Könyv- és Hírlap Külkereskedelmi Vállalatnál (1389 Budapest, 62, P. O. B. 149 Bankszámla: 218-10-990) vagy annak külföldi képviseleteinél és bizományosainál.

The Acta Agronomica publish papers in English on agronomical subjects, mostly on basic research.

The Acta Agronomica appear is one volume (four issues) a year.

Manuscripts should be addressed to:

Acta Agronomica
H-2462 Martonvásár, Postafiók 19.

The rate of subscription is $ 32.00 a volume.

Orders may be placed with "Kultúra" Foreign Trade Company for Books and Newspapers (1389 Budapest 62, P. O. B. 149 Bank Account No. 218-10-900) or with representatives abroad.

ПЕРЕДВИЖЕНИЕ И НАКОПЛЕНИЕ МОРФАКТИНА, МЕЧЕННОГО C^{14}, В ПЛОДОВЫХ ПРУТИКАХ

Р. ШТЭССЕР, Т. БУБАН, Ф. МЮЛЛЕР

Листья обрезанных плодовых прутиков яблонь (сорт Йонатан) были обработаны хлор-флуренолом, меченным C^{14}, который является действующим агентом морфактина, обозначенного EMD—IT 3456. В подобных условиях опыта активный раствор был введен и в углубление плодоножки.

Практически найдено равномерное распределение в тканях листьев, но в губчатой паренхиме активность была немного слабее. В поперечных разрезах листовых черешков часто бывает, что флоэма проявляет более сильную маркировку. Инкорпорация, наблюдаемая в тканях верхушечной почки плодовых прутиков, является относительно равномерной. Инкорпорация внутри семяпочки как в интегументе, так и в эндосперме без сомнения выявлена. Выполненная одновременно макроавторадиография показала, что в течение пяти часов после обработки не наблюдалось передвижения в направлении зрелых листьев, находившихся напротив обработанных листьев. Использованный морфактин легко инкорпоруется и быстро транспортируется как в базипетальном, так и акропетальном направлениях.

ДЕЙСТВИЕ ЗАРАЖАЮЩЕГО МАТЕРИАЛА CLAVICEPS PURPUREA С РАЗНЫМ ЧИСЛОМ СПОР НА КОЛИЧЕСТВО СПОРЫНЬИ

Т. ШОШ

Проведены опыты для того чтобы установить, можно ли достичь большего урожая спорыньи при повышении числа спор в суспензии Claviceps purpurea. Обычно в практике применяется суспензия с 5×10^3 спор/мм^3. Эксперименты, проведённые в Ракошкерестуре, показали, что в опыте №1 урожай достоверно повысился при применении заражающего материала с числом спор 10×10^3/мм^3.

ИЗУЧЕНИЕ ПОТРЕБНОСТИ МИКРОЭЛЕМЕНТОВ И МЕСТА ПРОИЗРАСТАНИЯ БОРВИНКА (VINCA MINOR.)

ДЬ. ТЭЛДЬЕШИ, П. КАПОШИ

Авторы исследовали растения борвинка (Vinca minor L.) с 57 различных мест произрастания, а также почвы с этих мест с целью изучения потребности питательного вещества и места произрастания растений. Установлено, что на месте естественного произрастания растений доминируют кислые лесные почвы, которые в основном характеризуются низким числом стабильности гумуса и высоким содержанием подвижного железа и марганца. Для растения характерно содержание железа, марганца и меди, превышающее среднее естественного растительного покрова, что сочетается с меньшей концентрацией натрия и молибдена, чем среднее. Затруднения полевой культуры происходят от того, что растение, посаженное на чернозёмной почве, не может удовлетворить особую потребность в питательных веществах и вследствие пониженной устойчивости вымирает в результате грибных заболеваний. На почвах, содержащих много кальция, количество подвижного марганца должно быть повышено при помощи удобрения сернокислого аммония и диводородного фосфорнокислого аммония, поглощение значительного количества элемента которого является одной из характерных черт хемотаксономии Vinca minor.

ДВА НОВЫХ ЕСТЕСТВЕННЫХ РАСТЕНИЯ—ХОЗЯИНА МОЗАИЧНОГО ВИРУСА РЕПЫ В ВЕНГРИИ

Н. ЮРЕТИЧ, Й. ХОРВАТ, Д. МАМУЛА, В. Х. БЕСАДА, Л. БЕЦНЕР

Настоящая статья сообщает о спонтанном появлении мозаичного вируса репы (**TuMV, syn.: cabbage black ring virus,** x/x : x/x : E/E : S/Ap) у репы *(Brassica rapa* L. var. *rapa)* и капусты *(Brassica oleracea* L. var. *capitata)* в Венгрии. Вирус был идентифицирован на основании пробы растительной реакции и ряда растений-хозяев, серологии, электронной микроскопии, трансмиссибильности тли, включения тела и физических свойств. Два изученных изолята мозаичного вируса репы (HS и K30) по-видимому являются подобными в реакциях растений-хозяев к TuMV-JN и TuMV, все изолированные из цветной капусты и горчицы в Венгрии (Horváth *et al.* 1975). Электронная микроскопия показала извилистые нитевидные частицы, составляющие в среднем 730 нм по длине. Вирус был передан легко через *Myzus persicae* Sulz. Как HS так и K30 изоляты мозаичного вируса репы образовывали гранулярные цитоплазматические x-тела овальной или неправильной формы, часто содержащие кристальные иглы. Они имели следующие физические свойства: термическая точка инактивации 56—58°C, конечная точка разведения 2×10^{-3}—2×10^{-4}, долговечность *in vitro* 2—3 дня. Два изолята вируса были серологически родственны югославскому штамму мозаичного вируса репы, изолированному из капусты.

РЕЗУЛЬТАТЫ ИЗУЧЕНИЯ ЭПИДЕРМИСА У СОРТОВ ТАБАКА

Е. ПАНКУЧИ

Влияние внешних условий в значительной мере модифицирует формирование эпидермиса, имеющего тесную связь с окружением. Все важные параметры эпидермиса определенным образом реагируют на эффекты окружающих условий, в первую очередь на температуру. Изменение характерных анатомических признаков является очевидным и наблюдаемая тенденция одинакова. Число устьиц повысилось как на верхней, так и на нижней поверхностях листа. Форма устьиц изменилась менее, а форма клеток — более: длина клеток снижалась соответственно с поздним посевом, то есть клетки стали более округлыми. Пропорция устьиц наглядно изменилась в зависимости от времени посева у всех сортов. По показателю индекса стомы наблюдалось наибольшее различие между отдельными сортами и изменение в зависимости от времени посева оказалось более выравненным, тенденция изменений однообразной. Полученные данные распределяются по оптимальной кривой.

ОСНОВНЫЕ АМИНОКИСЛОТНЫЕ ФОСФАТЫ МИОФИБРИЛЛА, МИОЗИНА

1. Нахождение фосфогистидина в миозине и в миофибрилле

Ш. ФАЗЕКАШ, И. КАША, В. СЕКЕШИ-ХЕРМАНН

На основе предыдущей работы (Fazekas 1973, 1974) установлено, что миозин обладает определённым количеством фосфора, имеющего ковалентную связь с атомным весом 10—12 г. При исследовании его изолировано 4М гистидин-фосфата и в этой статье подробно излагаем условия изоляции. Perrie и его сотрудники (1973) изолировали и определили по одному серин-фосфату из двух light chain миозина с молекулярным весом 18 000; так выяснен источник фосфата, имеющего ковалентную связь с атомным весом 6 г. При проведении наблюдений установлено, что гистидин-фосфат происходит из heavy chain миозина и его пептиды гораздо более устойчивые, чем свободный фосфогитидин. По нашему предположению, в миозине находятся и другие фосфаты, имеющие ковалентные связи, но для изоляции их настоящий метод не пригоден. Удалось получить один фосфопептид из миозина или его heavy chain путём триптиназ-переваривания. Во многих случаях измерили прямым методом большее количество фосфатов, имеющих ковалентные связи, чем это упоминалось выше, но большинство из них были лабильными при гидролизе. Мы старались согласовать биологическое значение и роль гистидин-фосфатов с имеющимися литературными данными, и на основе этого придаём гораздо большее значение при сокращении мышцы гистидину или другим фосфатам, имеющим ковалентные связи, чем осуществление простой активности ATP-азы, Mg^{2+} или Ca^{2+}.

ACTA AGRONOMICA

ТОМ 25, ВЫП. 1—2

РЕЗЮМЕ

СУТОЧНОЕ КОЛЕБАНИЕ СОДЕРЖАНИЯ САХАРА В НЕКТАРЕ И ПЕРИОДИЧНОСТЬ СЕКРЕЦИИ В СЕМЕЙСТВЕ COMPOSITAE

Й. ПЕШТИ

Количественный анализ нектара, выделенного цветочными нектарниками изученных видов, показал, что секреция нектара имеет суточный ритм. Ритм колебания не зависит от факторов внешней среды. В одинаковых родах, или в пределах большого родственного круга (подсемейства) продолжительность колебания может быть одинаковой, но количественные максимумы по-видимому являются характерными особенностями вида. В подсемействе Liguliflorae во время выделения нектара обнаруживается только один максимум, и это имеет место в первой половине дня. У видов, принадлежащих к подсемейству Tubuliflorae, можно наблюдать два или три максимума в секреции нектара. Факторы внешней среды (почва, относительная влажность воздуха, температура и т. д.) изменяют количественный и качественный состав нектара. При повышающейся температуре и снижающейся относительной влажности воздуха повышается содержание сухого вещества нектара.

РОСТ, ПОТРЕБЛЕНИЕ КОРМА И УБОЙНАЯ ЦЕННОСТЬ БЫКОВ РЫНСЕ-ПЯТНИСТАЯ ВЕНГЕРСКАЯ ПОРОДА Х КАНАДСКИЙ ХОЛШТЕЙН ФРИЗ РЫНЦЕ-ПЯТНИСТАЯ ВЕНГЕРСКАЯ ПОРОДА Х ДЖЕРСИ

Ф. Х. Х. ФАРРАГ

Две одинаковые группы по14 новорожденных бычков были отобраны по принципу лучайности из кроссбредного стада рынсе-пятнистая венгерская порода ♀ х Канадский Холштейн Фриз(F_1) (группа I) и рынсе-пятнистая венгерская порода ♀ х Джерси ♂ (F_1) (группа II) с целью изучить их рост, потребление корма и убойную ценность. Обеим группам было предоставлено свободное содержание и кормились отдельно. Одни и те же порции были использованы и предоставлены для опытных животных в течение всего эксперимента. В конце опыта средний окончательный живой вес был 578,2 и 494,3 кг для I и II групп соответственно. Средний ежедневный привес в течение опытного периода был 1,171 и 0,994 кг для I и II групп, соответственно. Средние крахмальные эквиваленты, требующиеся на каждый килограмм ежедневного привеса, составили 3,94 и 4,12 кг, а также 697 и 731 г переваримого белка требовались на каждый килограмм привеса в I и II группах, соответственно.

В конце кормления и периода откорма десять животных из каждой группы были отселектированы по принципу случайности для изучения убойной ценности двух групп. Проценты покровов животных равны 60,25 и 58,60 для I и II групп, соответственно. Изучены коэффициенты корреляции для определения взаимосвязи между оценкой экстерьера животного, оценкой скелета процентом покрова животного и процентом жира двух кроссбредных групп. Только оценка экстерьера и процент покрова животного рынсе-пятнистая венгерская порода х и Джерси (II группа) коррелировали достоверно (0,59, $P < 0,05$).

ФИЗИОЛОГИЧЕСКОЕ ИЗУЧЕНИЕ ПЫЛЬЦЫ ГРУШИ

Й. НИЕКИ

В 1968, 1969 и 1970 годах у 6 диплоидных и 2 триплоидных сортов груши изучалось прорастание пыльцы в лабораторных условиях. В каждом сорте процент проросшей пыльцы проявлял значительные колебания по годам изучения. Качество пыльцы зависело от процесса редукционного деления клеток и климатических условий. Различия по годам по проценту проросшей пыльцы были связаны с периодом завязывания плодов и числом фертильных семян на плод. На основе процента прорастающей пыльцы сорта могут быть отнесены к трем группам: плохая (с плохо прорастающей пыльцой) — меньше 30 процентов, средняя — 30 — 60 процентов, хорошая — больше 60 процентов.

ОПРЕДЕЛЕНИЕ ПИТАТЕЛЬНОЙ ЦЕННОСТИ КОРМОВ ПУТЕМ НЕПОЛНОГО АНАЛИЗА НА ОСНОВАНИИ ВЗАИМООТНОШЕНИЯ ИХ ХИМИЧЕСКОГО СОСТАВА

Е. СЮЧ, М. КЕРЕСТЕШ, И. БОДА, И. ТИЛДИ

На 219 образцах кукурузного силоса, 570 — люцерного сена, 262 — зерновой муки 120 — пшеничных отрубей и 87 — экстрактированной подсолнечной муки авторы исследовали взаимоотношение между сырым составом кормов, усвояемым сырым белком и крахмальным эквивалентном, высчитанными традиционными методами на основании полного анализа. По выводам авторов, взаимоотношение химического состава кормов может дать теоретическую основу для калькуляционной системы, которая позволит определить питательную ценность кормов в пределах данной ошибки на основании неполного анализа с использованием регрессионного уравнения, представленного в статье.

ВЛИЯНИЕ CCC И КОЛИНА НА РОСТ ПОБЕГОВ И ОБЩЕЕ СОДЕРЖАНИЕ УГЛЕВОДА И СЫРОГО БЕЛКА В ДВУХЛЕТНИХ СЕЯНЦАХ ДИКОЙ ГРУШИ

Д. ШУРАНИ

Двухлетние сеянцы груши 6-го мая и 20-го мая были обработаны CCC (Clor Colin Clorid) и колином в концентрациях 0, 500, 1000, 2000 и 4000 ппм. Между растениями, обработанными CCC и колином, обнаружена разница как по высоте растений, диаметру корневой шейки, длине междоузлий, так и по общему содержанию углевода и сырого белка стебля и корня. На основании 4 обработок было выявлено достоверное повышение общего количества углевода в стебле и содержание сырого белка в корне. Автор пришёл к заключению, что сокращение молодой морфологической стадии дикой груши при помощи CCC гораздо более труднорешимая проблема, чем можно было справедливо ожидать на основании анализа однолетнего сеянца.

ВЛИЯНИЕ РАЗНЫХ УРОВНЕЙ БЕЛКА РАЦИОНА НА УСВОЯЕМУЮ ЭНЕРГИЮ ЯГНЯТ

Ш. А. МАХМУД, М. ТЕЛЕКИ

Усвояемая энергия была определена при изучении переваривания у ягнят, корм которых имел разные уровни рационного белка в течение их роста. Процент усвояемой энергии уменьшался при повышении уровня белка рациона.

ANDOR JÁNOSSY

1908—1975

Hungarian agricultural science has suffered a great loss. Dr. Andor Jánossy academician, titular university professor, director of the Institute of Agrobotany, president of the Association of European Plant Breeding Researchers, holder of many decorations has died. His decease came unexpectedly early, he could have continued to live and work, but his strength was not sufficient to fight his sudden illness.

He was born on 20 August 1908 at Szombathely, where his father was an officer at the orphans' court. He spent most of his childhood at Kemenesalja, at the foot of the Alps, where the rich scenery and well developed agriculture soon made him turn his attention to plant growing.

He completed his secondary school studies at Szombathely, then was admitted to the Agricultural Department of the Faculty of Economic Sciences at Budapest University where he graduated in 1930. In 1933 he obtained a doctor's degree in agricultural sciences at the same university.

Having acquired his diploma he spent six years in practice, working on various estates (Nyírbátor, Mezőhegyes) and widening his knowledge, then for a short time he was employed at the State Farm Inspectorate. His first permanent post was in the Plant Production Office, and seed supply in Hungary; from 1945 to 1950 he was in charge of this Office. Here he came into close contact with plant breeding, since the supervision of the propagation of improved varieties fell within the scope of authority of the Office. Frequent consultations with Rudolf Fleischmann and the regular observation of his work were very fruitful for Jánossy, as Fleischmann was a recognized plant breeder well-known both in Hungary and abroad.

All this had a great effect on Jánossy and determined the course of his further work. It was probably then that he decided to utilize his talents in the service of plant breeding and variety testing. This was the turning point of his life. He became head of department at the Institute of Plant Breeding in 1950, then in 1952 was appointed director of the National Institute for Seed Testing. But he did not work long in this famous institution. In 1953 a Plant Variety Testing Department was organized within the framework of the Horticultural Research Institute, and Jánossy was charged with its management. It soon became clear that the Department could no longer be kept within the Horticultural Research Institute (it had an independent scope of authority anyway), and the National Institute for Plant Variety Testing came into existence in Budapest by ministerial decree in 1963, with Andor Jánossy as its director. Jánossy wrote the following about the tasks of the institute in the 1953 Year-Book: "*Our tasks* have been determined by ministerial decree as follows: 1. the execution of national micro-plot and farm-scale variety trials; 2. the control, direction, etc. of activities in connection with plant variety certification; 3. the investigation of the biological and morphological properties of plant varieties, and, on the basis of the results, the description of the domestic and foreign plant varieties under cultivation; 4. research work concerning the methodology of field trials; 5. the collection and classification of fruit, vine, outdoor ornamental and medicinal plant varieties, the organization of new sites for such variety trials, research into variety evaluation and its methods; 6. agrotechnical research in connection with the question of variety; 7. the administration of the Plant Variety Qualification Council". It was worth quoting all this word for word because the taks also reflect Jánossy's ambitions, which he wished to realize to the full in the new institute.

It is to his everlasting merit that he organized the National Institute for Plant Variety Testing with considerable talent and directed its work at a highly up-to-date level. The "Fajtakísérletek Eredményei" (Results of Variety Trials) published from year to year are like milestones demonstrating the development of Jánossy's activities and his outstanding capabilities as a leader. During his activity as director of the Institute he was asked on several occasions to take over the direction of the Research Centre of the Ministry of Agriculture. Jánossy attended with great energy and skill to his work too.

In 1956 he was awarded the degree of candidate of the agricultural sciences.

Jánossy was the director of the National Institute for Plant Variety Testing until 1959. Then he was given a special assignment. He was commissioned to organize and direct the National Institute of Agrobotany at Tápiószele. The government decree No. 60/1958 prescribed the duties of the new Institute as follows: " . . . to collect and maintain Hungarian and foreign varieties of cultivated plants, and carry out taxonomical, botanical, physiological, biochemical and pathological studies on them".

Jánossy had the following to say about the history of his new sphere of activity: "My attention was first called to the importance of collecting and studying the basic material of plant breeding 30 years ago (1939 !) by my scientific master Rudolf Fleischmann. It was he who made me acquainted with Vavilov's works, with the theory of gene centres. However, it was only after the liberation, in 1948, — within the framework of the former Plant Production Office — that an opportunity arose to collect the local wheat varieties of the Hungarian basin by searching over large areas". Jánossy had long been enthusiastic about the task of collecting and maintaining the old varieties. Besides his other duties he did not fail to carry out successful in this field, too, in which real possibilities were offered to him at the new Institute. From then on he worked in this field untiringly until the end of his life.

When it was first established the new Institute was supplied with very modest funds. Through Jánossy's indefatigable activity, however, it soon flourished, and became internationally known and appreciated. All this was to the credit of Jánossy's talent and skilful leadership.

In 1966 he became Academic Doctor of the Agricultural Sciences, and in 1970 — as a recognition of his merits — he was elected member of the Hungarian Academy of Sciences.

During his busy life Jánossy wrote numerous books and papers. His papers were published in Hungarian and foreign periodicals. He played an active part at scientific meetings and conferences both in Hungary and abroad and received wide recognition. As the highest government recognition he was awarded the gold medal of the Order of Labour (1965), and was decorated with the Rudolf Fleischmann and László Baross medals too. The esteem in which he was held abroad is demonstrated by the fact that he was elected president of the Association of European Plant Breeders.

He also carried out highly valuable plant breeding work. He produced several state certified varieties which are widely grown and appreciated in Hungary. He was specially interested in the clovers, of which lucerne and red clover were the favourite subjects of his scientific studies and breeding work. His tetraploid red clover achieved world-wide success.

Finally, we must speak of Jánossy the man. He was a man with extremely pleasant manners, always ready to help and to promote the affairs of young people. His human attitude was characterized by a general goodwill which made friends for him all over the world. He was always ready to teach the young. As a titular university professor he delivered lectures at the University of Agricultural Sciences in Debrecen and elsewhere several times a year. He was a member or even honorary member of numerous scientific bodies and associations both in Hungary and abroad. He was readily elected to any organization; his pleasant personality attracted everybody. He was a member of the editorial boards of both Hungarian and foreign periodicals, where his excellent qualities were a great asset in the responsible work of editing.

It was with heavy hearts that his many friends took their final leave of him on May 4th 1975, when his sudden death wrested him away from his friends and loved ones. We shall guard his memory for ever.

Gy. Mándy

Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 5—17 (1976)

DAILY FLUCTUATIONS IN THE SUGAR CONTENT OF NECTAR AND PERIODICITY OF SECRETION IN THE COMPOSITAE

By

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The quantitative analysis of nectar secreted by the floral nectaries of the examined species shows that nectar secretion has a daily rhythm. The rate of fluctuation is independent of environmental factors. Within the same genus or larger relationship group (subfamily) the periods of daily fluctuation can be identical, but the quantitative maxima seem to be properties characteristic of the species. In the subfamily *Liguliflorae* nectar secretion has a single maximum and that before noon. In species belonging to the subfamily *Tubuliflorae* two or three maxima of nectar secretion have been pointed out. The environmental factors (soil, relative humidity, temperature, etc.) change the qualitative and quantitative composition of the nectar. With increasing temperature and decreasing relative humidity the dry matter content of the nectar will become higher.

Introduction

Attention was first called to the daily fluctuation of nectar secretion by Bravais (1842), Liebig (1846), Caspary (1848), Bonnier (1878). The number of bees visiting a plant species was found to change during the day according to the quantity of the secreted nectar. Dolgova (1928) studied the intensity of visiting by bees in various species and found that *Echium vulgare* had the most visitors around 3 p. m. Frisch (1934) completed this observation by pointing out that higher concentrations of sugar solution attracted more bees than lower ones.

Hambleton (1925) studied the fluctuation within 24 hours of the activity of a bee hive and arrived at the conclusion that in *Liriodendron* a secretion minimum occurred at about 2 p. m. Namely, during the experimental period only this plant was visited by the bees. Subsequent observations by Marvin (1933) did not, however, confirm this supposition.

Park (1929) studied the fluctuation of sugar concentration during 24 hours in *Tilia americana* and found that the concentration reached a maximum immediately after 12 o'clock, and from 4 p. m. sharply decreased.

The daily fluctuation of the sugar content of nectar is in connection with the relative air humidity. According to Park (1929) this dependence is higher in the *Tilia* species and the *Asclepias syriaca* than in *Tecoma radicans*. The latter has much more closed flowers, so the relative humidity in their inside is more constant. In the genus *Tilia* BEUTLER (1930) found the secretion to be the most intensive in the night hours. Other plants, as e.g. the *Asclepias* and *Fritillaria* secrete the largest quantity of nectar during the day, the *Tropeolum* in the late after-noon and evening hours. The secretion of *Fritillaria imperialis* is the most intensive in the early after-noon, and the sugar content of its nectar is maximum 10 per cent. On rainy, cool days the secretion is intensive while in sunny weather the nectar amount decreases. In concentration, however, there is but a slight difference. When the secretion starts the sugar concentration is 2—3 per cent; later it increases to 8—10 per cent, then decreases to 5 per cent, and finally the nectar dries up.

LUNDER (1945) pointed out in *Calluna* that in the late morning hours the sugar concentration rose from 44 to 60 per cent, remained unchanged until 6 p. m., then decreased again.

In studying the daily fluctuation the age of the flower must also be taken in consideration. BRAVAIS (1842), BONNIER (1878), HAUPT (1902), BEUTLER (1930), EWERT (1939), BOETIUS (1948), FAHN (1949), PANKRATOVA (1950), FREY-WYSSLING—AGTHE (1950) described their investigations on this subject. In general, the young and old flowers produce less and lower concentration nectar than the sexually mature flowers. Moreover, the glands of old flowers resorb the nectar (FREY-WYSSLING—AGTHE 1950).

Our investigations made on selected species of the family *Compositae* were conducted on two lines. On the one hand, we studied the daily fluctuations of nectar production with the purpose of obtaining detailed information on the functional periodicity of nectaries and on the related characteristics of the species. On the other hand, we examined the influence of air humidity and temperature on the dry matter content and quantity of nectar under the same soil conditions.

Material and Method

The daily fluctuation of nectar production was studied in 21 species living in natural habitat, of which 17 belonged to the subfamily *Tubuliflorae* and 5 to the *Liguliflorae.* The examined plants of each species grew in three different habitats where the soil- and microclimatic conditions were varied. In the evaluation averages obtained from these data were taken in consideration. Samples were taken every two hours; in species belonging to the subfamily *Tubuliflorae* between 7 a.m. and 5 p.m. while in those belonging to the *Liguliflorae* between 7 a.m. and 1 p.m. Namely, in the species of the two subfamilies nectar secretion showed a typical activity in the above intervals. Between two occasions of sampling the flowers were covered by a net. For each measuring the sample was taken from 100 flowers. For serial measuring samples were taken partly successively on the same day, partly on successive days at the same time. By the latter method we wished to eliminate the disturbing effect of the changing meteorological factors.

Experiments aimed at studying the effect of relative air humidity and temperature were carried out with 8 species. The experimental plants were grown on the same soil whose important physical and chemical properties had previously been thoroughly studied. Samples were collected every two hours between 7 a.m. and 5 p.m. and the temperature and relative humidity of the air were recorded at the same time. The dry matter content of the nectar sample was determined by a refractometer. The numerical results of repeated measurements were averaged and the data thus obtained included in tables, or plotted.

Results

I. Periodicity of nectar secretion. The nectar production of the examined species is shown in Table 1. Each figure represents the mg nectar production at the time of sampling, on the basis of averaged serial measurements of samples taken from a hundred flowers. Species from 1 to 17 belong to the subfamily *Tubuliflorae*, and those from 18 to 22 to the *Liguliflorae*. For species belonging to the *Tubuliflorae* the daily changes of nectar production are plotted in Figs 1 and 2 where the production curves of the examined plants are illustrated. These figures reflect two main characteristics concerning the periodicity of nectar secretion: first, secretion has two or three maxima a day, secondly, the maxima generally follow one another with about four hours intervals.

Table 1

Nectar production in the examined species

Name	Samples taken g/hour					
	7 a.m.	9 a.m.	11 a.m.	1 p.m.	3 p.m.	5 p.m.
1. *Arctium minus* Bernh.	14.57	14.95	16.07	12.57	17.98	3.75
2. *Arctium lappa* L.	14.00	18.08	23.60	14.11	16.04	14.08
3. *Centaurea cyanus* L.	11.05	16.37	5.07	7.41	6.16	13.76
4. *Centaurea cyanus* L. cv. Fl. Pl. *carminea*	0.66	12.41	3.50	5.30	0.45	1.00
5. *Centaurea jacea* L.	6.20	9.58	6.84	9.52	5.62	11.92
6. *Centaurea pannonica* Simk.	5.89	7.39	6.35	14.24	7.75	8.64
7. *Centaurea micranthos* Gmel.	50.00	13.39	14.69	7.79	15.28	11.33
8. *Chrysanthenum carinatum* "Radiatum"	2.73	3.21	4.12	3.02	8.38	3.75
9. *Cosmos bipinnatus* Cav.	11.58	13.25	9.26	17.34	13.08	5.62
10. *Coreopsis grandiflora* Hook.	6.65	23.70	2.89	6.25	5.50	2.56
11. *Coreopsis tinctoria* Nutt.	1.16	3.08	0.98	1.27	1.20	0.64
12. *Echinops commutatus* Jur.	45.39	66.34	88.66	23.46	27.81	53.45
13. *Helianthus annuus* L.	19.88	57.03	13.88	15.10	14.80	12.65
14. *Rudbeckia hirta* "Meine Freude"	5.24	4.44	4.18	6.07	5.40	0.91
15. *Tagetes patulus* L.	5.43	25.17	23.80	10.87	12.55	8.50
16. *Zinnia angustifolia* H. B. et Kth.	53.71	12.77	88.08	86.25	48.33	9.53
17. *Zinnia elegans* Jacq.	25.62	14.82	52.63	48.94	11.68	3.62
18. *Cichorium intybus* L.	0.89	4.07	2.43	0.62	0.00	0.00
19. *Crepis setosa* Hall.	2.61	7.17	7.75	2.53	0.00	0.00
20. *Lactuca sativa* L.	0.84	2.34	3.69	1.28	0.00	0.00
21. *Leontodon autumnalis* L.	2.23	3.44	1.60	0.88	0.00	0.00
22. *Leontodon hispidus* L.	4.13	5.35	6.82	1.24	0.00	0.00

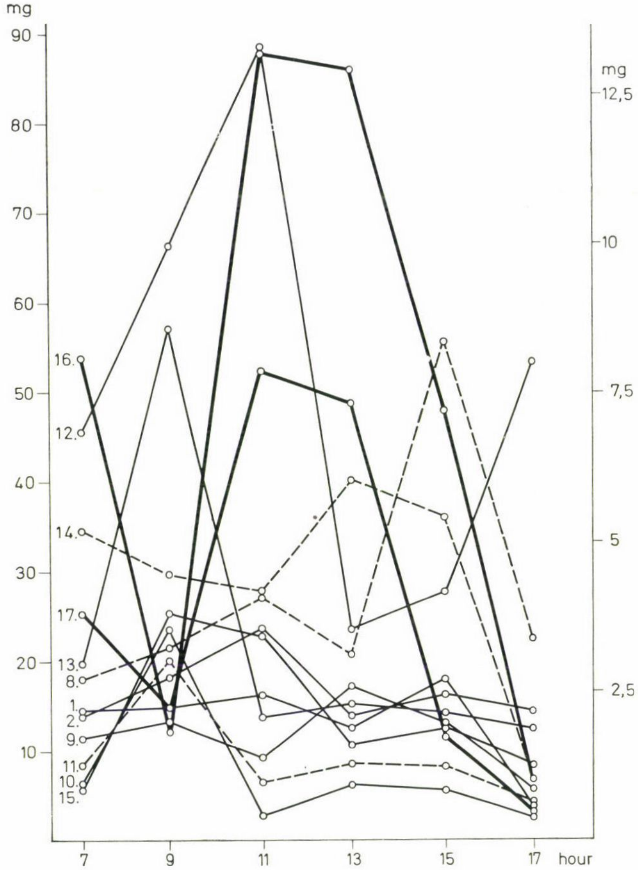


Fig. 1. Production curves of *Tubuliflorae* species showing two maxima in nectar production. (The values of curves drawn with a broken line are on the right side axis.) 1. *Arctium minus* Bernh. 2. *Arctium lappa* L. 8. *Chrysanthemum carinatum* "Radiatum". 9. *Cosmos bipinnatus* Cav. 10. *Coreopsis grandiflora* Hook. 11. *Coreopsis tinctoria* Nutt. 12. *Echinops commutatus* Jur. 13. *Helianthus annuus* L. 14. *Rudbeckia hirta* "Meine Freude". 15. *Tagetes patulus* L. 16. *Zinnia angustifolia* H. B. et Kth. 17. *Zinnia elegans* Jaqu

Of the species secreting with two maxima (Fig. 1) in *Zinnia elegans* Jacq. and *Zinnia angustifolia* H. B. et Kth. both maxima occur before noon. In *Arctium minus* Bernh., *A. lappa* L., *Chrysanthemum carinatum* "Radiatum", *Cosmos bipinnatus* Cav., *Coreopsis tinctoria* Nutt., *C. grandiflora* Hook., *Echinops commutatus* Jur., *Helianthus annuus* L., *Rudbeckia hirta* "Meine Freude" and *Tagetes patulus* L., on the other hand, one maximum occurs before noon, and one in the afternoon. As to the fluctuation of nectar secretion the species show remarkable similarities and differences. The secretion period is almost the same in *Helianthus annuus* L., *Coreopsis tinctoria, Coreopsis grandiflora, Cosmos bipinnatus, Tagetes patulus*. The production curves of *Helianthus annuus, Coreopsis tinctoria, Coreopsis grandiflora* and *Cosmos bipinnatus* are

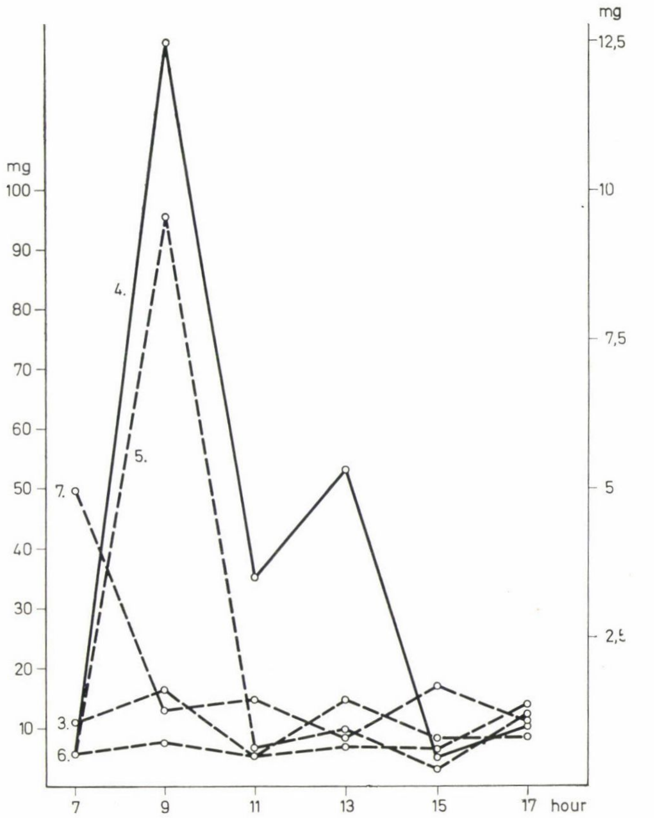


Fig. 2. Production curves of *Tubuliflorae* species showing three maxima in nectar production. 3. *Centaurea cyanus* L. 4. *Centaurea cyanus* L. cv. Fl. Pl. *carminea*. 5. *C. jacea* L. 6. *C. pannonica* Simk. 7. *C. micranthos* Gmel. Graphs marked with broken lines are shown on the left side axis

nearly identical. After the first maximum at 9 a. m. the second maximum — which is negligible compared to the first one — occurs at 1 p. m.

The production curve of *Tagetes patulus* differs from the former ones inasmuch as the higher, morning maximum is shown at 11 a. m. while the afternoon maximum at 3 p. m. Thus in the maxima a two hours shift can be observed compared to the former species, but the second maximum — here too — sets in with a four hours interval.

In the two species of the *Zinnia* genus *(Z. angustifolia* and *Z. elegans)* only the amounts of secretion are different, the periodicity is the same. Even the quantitative differences change proportionately during the day, the course of the production curve is perfectly identical in the two *Zinnia* species. Both maxima occur before noon. After the higher maximum at 11 a. m. secretion is not reduced to a minimum. Secretion takes place between 11 a. m. and 1 p. m. almost with the same intensity. The minimum sets in gradually.

The secretion periods of *Chrysanthemum carinatum* "Radiatum", *Arc-*

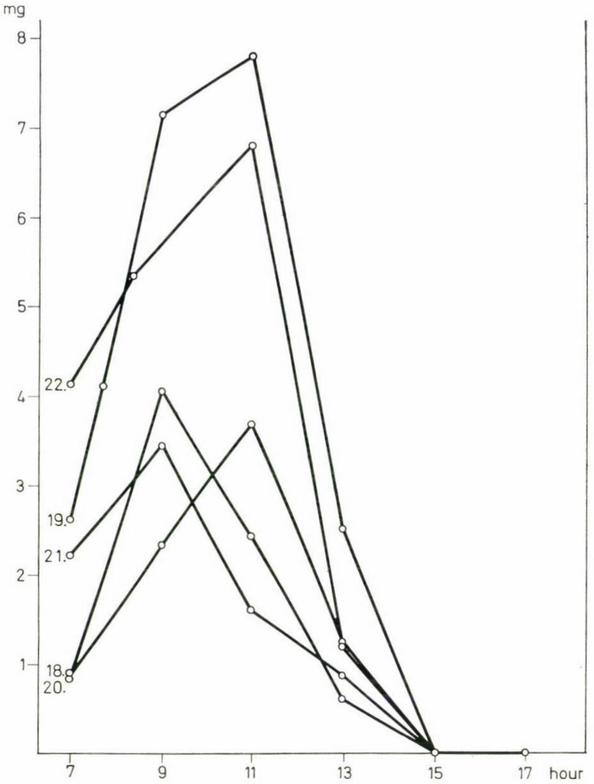


Fig. 3. Production curves of species belonging to the subfamily *Liguliflorae.* 18. *Cichorium intybus* L. 19. *Crepis setosa* Hall. 20. *Lactuca sativa* L. 21. *Leontodon autumnalis* L. 22. *Leontodon hispidus* L.

tium minus, *Arctium lappa*, *Echinops commutatus*, *Rudbeckia hirta* "Meine Freude" are similar. In nectar production the two *Arctium* species and the *Echinops commutatus* are superior to the *Chrysanthemum carinatum* "Radiatum", but the fluctuation of secretion and the proportion of the secreted nectar are almost the same in them. By 5 p. m. the nectar production is reduced to a minimum in *Arctium* and *Chrysanthemum*, and increases in *Echinops commutatus*. The production curve of *Rudbeckia hirta* "Meine Freude" is the perfect opposite of that of *Echinops commutatus*. In *Echinops* the maxima occur at 11 a. m. and 7 p. m., while in *Rudbeckia* at 7 a. m. and 1 p. m. The production curves clearly show that even a minimum production of *Echinops* exceeds by far the maximum nectar production of *Rudbeckia*.

Of the species secreting with three maxima (Fig. 2) the *Centaurea micranthos* Gmel. shows two maxima before noon and one in the afternoon while in *Centaurea cyanus* L., *C. cyanus* L. cv. Fl. Pl. *carminea*, *C. jacea*, *C. pannonica* one maximum occurs before noon and two occur in the afternoon.

In *Centaurea cyanus*, *C. cyanus* cv. Fl. Pl. *carminea*, *C. pannonica* and *C. jacea* the three maxima occur at the same time (9 a. m., 1 and 5 p. m.). In *Centaurea micranthos* the maximum sets in two hours earlier. The two closely related species *C. jacea* and *C. pannonica* have turned out to be very similar both in the periodicity of secretion and the amount of the secreted nectar. The production curves of *Centaurea cyanus* and *C. cyanus* cv. Fl. Pl. *carminea* are perfectly identical but it is remarkable that the ornamental variation produces essentially less nectar.

The species of the subfamily *Liguliflorae* substantially differ from those of the *Tubuliflorae* in the periodicity of nectar secretion. Owing to the typical nastic motions of flowers only a single maximum appears here. The flowers are open only before noon, so this single maximum occurs at 9 and 11 a. m., respectively. In these species nectar production could only be examined between 7 a. m. and 1 p. m. (Fig. 3).

The production curves drawn from the measuring results are very similar in these species. This can be attributed to the above mentioned characteristic of the subfamily. The maximum values are always below the production of the *Tubuliflorae* species. The maximum occurs at 9 a. m. in *Cichorium intybus*, *Crepis setosa* and *Leontodon autumnalis*, and at 11 a. m. in *Lactuca sativa* and *Leontodon hispidus*.

II. Daily fluctuations in the sugar content of nectar. Changes in the dry matter content of nectar as a function of air humidity and temperature were studied in eight plants under identical soil conditions. Samples were taken every two hours between 7 a. m. and 5 p. m. The soil conditions were as follows:

Physical soil type: clay loam
Sticky point by Arany: 47
pH in distilled water: 7.4 — alkalescent
pH in potassium chloride: 7.0 — neutral
Lime condition ($CaCO_3$): 2.31 mg — satisfactory

Nutrient supply:

Humus content: 4.02 per cent — good
Total nitrogen: 0.24 per cent — medium
Available P_2O_5: 40.00 mg/100 g — very good
Available K_2O: 59.80 mg/100 g — very good

Microelement content: mg/kg

Zn	B	Mo	Fe	Cu	Mn
48.00	1.90	0.16	25.71	0.50	62

Table 2

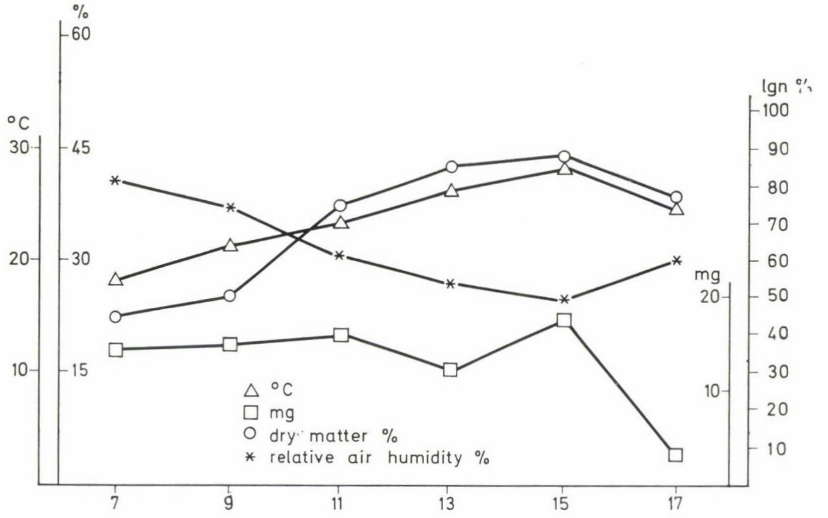
Effect of temperature and relative air humidity on the dry matter content and quantity of nectar

Name	Time	Temperature °C						Relative air humidity					
		7	9	11	1	3	5	7	9	11	1	3	5
		a.m.			p.m.			a.m.			p.m.		
Arctium minus (Hill). Bernh.	1971 22 August	18	21	23	26	28	25	81	75	61	53	49	60
Centaurea cyanus L.	1972 1 July	18	19	20	20	21	21	87	86	77	73	70	60
Centaurea jacea L.	1971 11 August	18	22	25	22	20	18	72	68	48	57	56	64
Centaurea pannonica (Heuff)Simk	1971 11 August	18	22	25	22	20	18	72	68	48	37	56	64
Cichorium inthybus L.	1972 29 July	20	20	21	22	—	—	93	91	89	85	—	—
Crepis biennis L.	1972 16 July	19	21	25	26	—	—	96	85	65	64	—	—
Solidago giganthea Ait.	1971 4 August	23	25	27	28	29	25	75	64	56	46	45	50
Zinnia elegans Jacq.	1971 17 August	17	19	23	25	25	20	70	60	45	41	41	59

Name	Dry matter %						Nectar weight 100 fl/mg					
	7	9	11	1	3	5	7	9	11	1	3	5
	a.m.			p.m.			a.m.			p.m.		
Arctium minus (Hill). Bernh.	22	25	37	42	44	38	14.57	14.45	16.07	12.57	17.98	3.75
Centaurea cyanus L.	16	20	22	27	34	35	11.05	16.07	5.07	7.41	6.16	13.76
Centaurea jacea L.	22	54	56	35	34	31	6.2	9.58	6.84	9.52	5.62	11.92
Centaurea pannonica (Heuff)Simk	27	45	57	37	34	31	5.89	7.39	6.35	14.24	7.75	8.64
Cichorium inthybus L.	18	30	20	—	—	—	0.85	2.06	3.81	—	—	—
Crepis biennis L.	13	25	32	15	—	—	2.75	2.65	2.13	1.11	—	—
Solidago giganthea Ait.	0.0	40	51	63	50	40	0.00	1.71	2.84	3.73	3.40	2.60
Zinnia elegans Jacq.	29	30	33	39	31	10	25.62	14.82	52.63	48.94	11.68	3.62

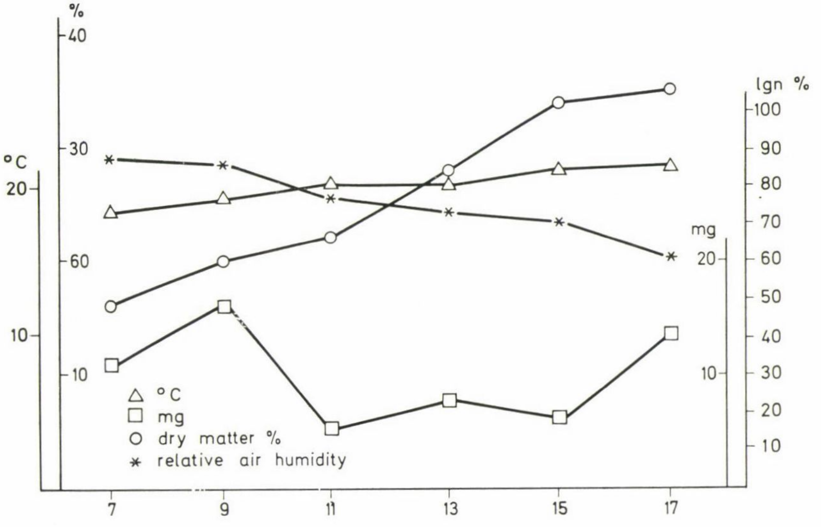
The numerical data of the experiments are averaged in Table 2. It shows the quantitative effects exerted by the temperature and relative air humidity on the trends of the dry matter content and quantity of nectar.

The graphic representation of the numerical data (Figs 4—11) clearly shows the mentioned correlations for the individual species. The increase of temperature and decrease of relative humidity result in an increase in the



1. *Arctium minus* (Hill.) Bernh.

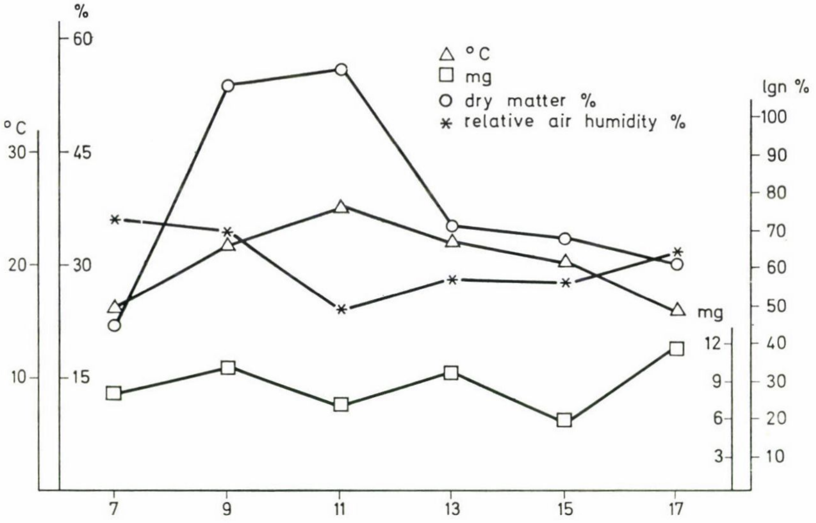
Fig. 4. Correlations of the percentage dry matter content and quantity of nectar with the temperature and relative air humidity



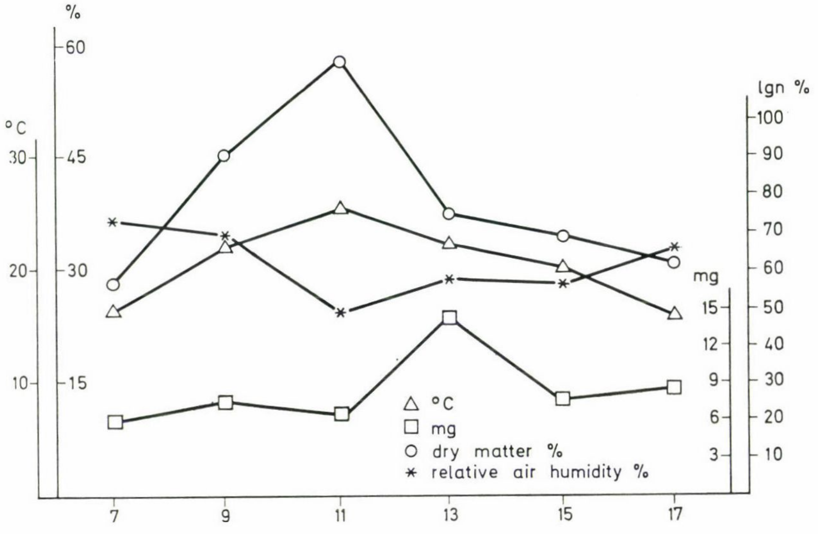
2. *Centaurea cyanus* L.

dry matter content of the nectar. Dry matter content generally reaches a maximum when the absolute values of the two factors are close to each other.

The mg values of the quantity of nectar produced show that the periodicity of secretion is not influenced by the environmental factors considered.

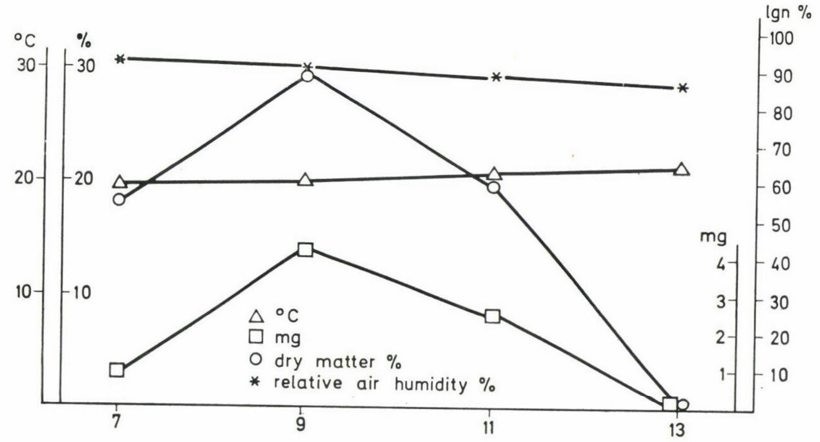


3. *Centaurea jacea* L.

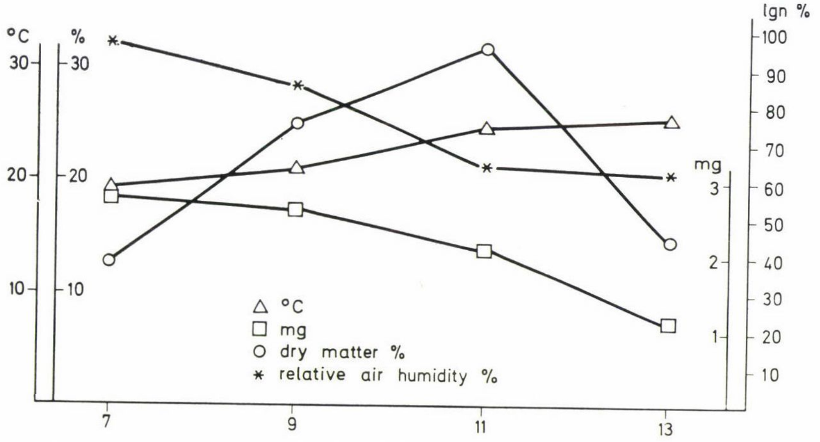


4. *Centaurea pannonica* (Heuff) Simk.

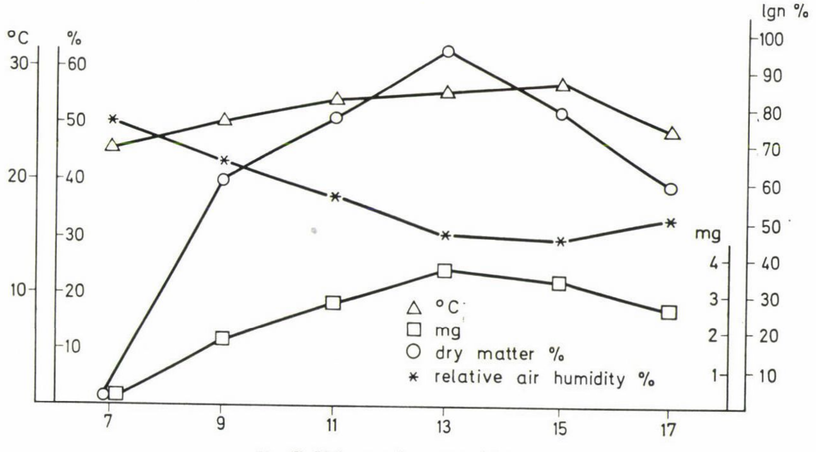
For example, in *Arctium minus* maximum production and dry matter percentage are found with an increasing temperature and decreasing humidity. In the case of *Centaurea pannonica* with the typical minimum secretion activity a maximum dry matter content was shown due to the modifying effect of the environmental factors.



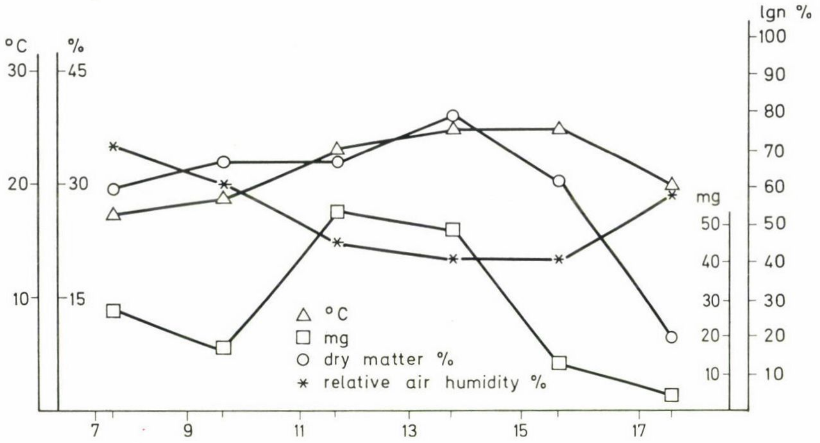
5. *Cichorium intybus* L.



6. *Crepis biennis* L.



7. *Solidago gigantea* Ait.



8. *Zinnia elegans* Jacq.

Conclusions

From our investigations made in different parts of the day we have arrived at the conclusion that in species belonging to the family of *Compositae* the floral nectar secretion and with it the function of glands are periodical processes with characteristic daily rhythms. This conclusion agrees in essentials with earlier results found in the literature relative to species belonging to other families. Our experiments suggest that this periodicity is of endogenous character since its peculiar rhythm, in some respects characteristic of the species too, is not essentially influenced by environmental factors. From this point of view the indifference of the soil- and microclimatic conditions, mainly of the temperature and relative humidity, is remarkable.

The periodicity of the secreting activity of the nectaries is at the same time highly different in the *Tubuliflorae* and *Liguliflorae* subfamilies. Besides the morphological differences this phenomenon seems — from a taxonomic point of view — to be a physiological characteristic of the subfamily. In the subfamily *Tubuliflorae* the nectar secretion has — characteristically of the species — two or three maxima a day between 7 a. m. and 5 p. m., which generally follow one another with a four hours interval. After the highest glandular activity the process of secretion generally slows down to a considerable extent. In the subfamily *Tubuliflorae*, on the other hand, only one maximum occurs, and that always before noon, between 9 and 11 a. m. This circumstance may be in close connection with the characteristic nastic motions of flowers in the *Tubuliflorae* species, which are responsible for these flowers being open only before noon.

From the quantitative results of our experiments it can be established, however, that apart from the periodicity the amount and the percentage of the sugar content of the produced nectar depend on exogenous, environmental factors, first of all on the temperature and relative air humidity. The increase of temperature and decrease of relative humidity cause an increase in the sugar content of the secreted nectar.

Summing up what has been said we arrive at the general conclusion that in the *Compositae* family the floral nectar secretion is a function of endogenous cytological processes which have specific endogenous daily rhythms. The quantity and composition — mainly the sugar content — of the secreted nectar depend, on the other hand, upon environmental factors.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 19—26 (1976)

GROWTH, FEED CONSUMPTION AND SLAUGHTER VALUE OF HUNGARIAN FLECKVIEH×CANADIAN HOLSTEIN FRIESIAN AND HUNGARIAN FLECKVIEH×JERSEY BULLS

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Two equal groups of 14 new-born bulls were selected at random from the cross-bred herd of Hungarian Fleckvieh ♀ × Canadian Holstein Friesian ♂ (F_1) (Group I) and Hungarian Fleckvieh ♀ × Jersey ♂ (F_1) (Group II) in order to study their growth, feed consumption and slaughter value. The two groups were loose-housed and fed separately. The same rations were used and offered ad libitum to the experimental animals throughout the experiment. At the end of the experiment, the average final live weights were 578.2 and 494.3 kg for Groups I and II, respectively. The average daily gains during the experimental period were 1.171 and 0.994 kg for Group I and Group II, respectively. The average starch equivalents required per each kg daily gain were 3.94 and 4.12 kg, also 697 and 731 g digestible protein were needed per kg gain for Group I and Group II, respectively. At the end of the feeding and fattening period, ten animals from each group were selected at random to study the slaughter value of the two groups. Dressing percentages were found to be 60.25 and 58.60 for Group I and Group II, respectively. The correlation coefficients for relationships among the live animal conformation score, carcass conformation score, dressing percentage and fat percentage of the two cross-bred groups were studied. Only the live-animal conformation score and the dressing percentage of Hungarian Fleckvieh × Jersey (Group II) was found to be significantly correlated (0.59, $P < 0.05$).

Introduction

In most countries where fresh meat is consumed, the dual-purpose breeds are its most important producers and suppliers. In the future the increase of beef production in these countries will be dependent on the rate of increase of the dual purpose herds.

Several cross-breeding experiments were carried out in Hungary, in order to improve the Hungarian Fleckvieh breed (Horn 1962, Bárczy *et al.* 1963, Nagy 1965, Szuromi 1969, Bozó *et al.* 1970). Different results were obtained regarding the growth and slaughter value of the new cross-bred animals.

Subjective live-animals and carcass conformation scores have long since been used extensively for evaluating all the slaughter animals of the pure-breds and cross-breds in Hungary. The aim of the present study was to compare the Hungarian Fleckvieh ×Canadian Holstein Friesian F_1 bulls with Hungarian Fleckvieh × Jersey crossbred F_1 bulls regarding growth, feed consumption and slaughter value and to study the accuracy of applying such qualification score for these cross-bred animals.

Material and Method

This experiment was carried out at the Szentegát State Farm (Hungary). 28 animals in two equal groups of newborn bull calves were selected at random from the cross-bred herds of Hungarian Fleckvieh ♀ × Canadian Holstein Friesian ♂ (H. F. × C-H. F.) (F_1), (Group I) and Hungarian Fleckvieh ♀ Jersey ♂ (H. F. × J.) (F_1), (Group II) respectively. The average live-weights at the starting of the experiment were 35.28 and 28.57 kg, respectively. The two groups of animals were kept loose and fed separately.

The rations fed to the experimental animals were of the same composition. The feeding program consisted of colostrum and mothermilk for ten days, followed by a commercial dry milk fed up to 102 days of the animals age. This milk was prepared by dissolving 8.9 per cent dry milk and 2.05 per cent lactin in 89.05 per cent water. During the suckling period calf-starter composed of 28 per cent barley, 30 per cent ground maize, 16 per cent soyabean meal, 4 per cent linseed meal, 2 per cent wheat bran, 1.6 per cent $CaCO_3$, 0.5 per cent NaCl, 0.4 per cent P_2O_5, 7 per cent yeast, 9 per cent dry milk powder, 1 per cent vitamin mixture, and 0.5 per cent micro mineral mixture was offered (ad libitum). After the weaning time, the concentrate rations were offered also (ad libitum) according to Table 1 till the end of the feeding and fattening period.

Table 1

Composition of concentrate rations used for feeding the two groups of experimental animals

Ingredients in %	Period A	Period B	Period C
Wheat	27.00	—	—
Ground maize	34.50	81.70	84.38
Extracted sunflower meal	8.80	8.80	2.70
Urea	2.70	2.40	2.05
Dried beet pulp	23.50	3.70	6.80
Vitamin mixture	0.55	1.30	0.70
Micro minerals mixture	0.55	0.50	1.35
$CaCO_3$	1.30	1.30	1.35
NaCl	1.10	0.50	0.70

Period A: From 77 to 209 days of animals age
Period B: From 210 to 353 days of animals age
Period C: From 354 to 466 days of animals age

From the starting of the experiment till the end of the seventh month of the animals age, alfalfa-hay was offered (ad libitum), then molass was fed till the end of the feeding period. The offered ration and the remaining feed were weighed daily, then the daily feed consumption was estimated and recorded.

The live body weight was recorded at the start of the experiment and regularly at the end of each month, the daily gain in weight was calculated during the experimental period.

At the end of the experiment, ten animals from each group were selected at random and transported to the slaughterhouse at Kaposvár. After fasting from feed for approximately 20 hours, the live weight and subjective scores for the live bulls' conformation were recorded according to the Hungarian method by a committee of two members of the Meat Research Institute and of the National Institute for Agricultural Variety Testing.

After slaughtering, the animals were skinned and dressed out, then the dressing percentage was immediately calculated on the basis of the hot carcass weight. The following organs and offals; head; hide; fore legs; lungs; heart freed from the large blood vessels; liver excluding the gall bladder; spleen; kidney fat and caul fat were weighed separately in each animal.

The cold carcasses were weighed on the following day after hanging for approximately 24 hours in a cold room (0 °C). Subjective scores were recorded for carcass conformation according to the Hungarian method, by a committee of two members of the Meat Research Institute and of the National Institute for Agricultural Variety Testing. The data were statistically analysed using mean values, standard errors, standard deviations, and simple correlation coefficients as suggested by SNEDECOR (1956). They were coded and punched on cards for analysis using the electronic computer of the Physiology Department, University of Veterinary Science, Budapest.

Results

The results of the present experiment concerning the growth rate, feed consumption and slaughter data of the two cross-bred groups of experimental animals are presented in Table 2. Faster growth was found in the animals

Table 2

Growth, feed consumption and slaughter data of the two cross-bred groups of the experimental animals

Traits	Group I HF♀×CHF♂		Group II HF♀×J♂	
	kg	%	kg	%
*Growth and feed consumption data**				
Initial live weight	35.28		28.57	
Feeding and fattening period	464	days	466	days
Final live-weight	578.22	117	494.28	100
Daily gain	1.171	118	0.994	100
Starch value/kg live gain	3.942	95.7	4.118	100
Digestable protein/kg live gain	0.697	95.3	0.731	100
*Slaughter data***				
Slaughter weight	550.00	100	467.00	100
Hot carcass weight	331.30		273.80	
Head	15.80	2.87	14.25	3.05
Hide	44.90	8.61	41.00	8.78
Four legs	9.82	1.78	8.51	1.82
Spleen	0.79	0.14	1.07	0.29
Liver	6.92	1.26	5.98	1.28
Heart	2.11	0.38	1.96	0.42
Lungs	5.78	1.05	5.07	1.09
Kidney	1.27	0.23	1.05	0.22
Kidney fat	13.51	2.46	12.28	2.63
Caul fat	8.34	1.57	8.87	1.90

* Growth and feed consumption data of Group I expressed as percentage of Group II
** Organs and offals of the two groups expressed as percentage of slaughter weight

crossed with Canadian Holstein Friesian (Group I) than in those crossed with Jersey (Group II).

The average daily gains were 1.171 and 0.994 kg throughout the experimental period for Groups I and II, respectively. The daily gain of Group I was 18 per cent higher than that of Group II.

The daily feed-consumption per 1 kg live weight gain were 3.942 and 4.128 kg starch value, 697 and 731 g digestible protein for H. F. × C-H. F. and H. F. × J., respectively. From these results it could be observed that the animals of Group I needed 4.3 per cent less starch value and 4.7 per cent less digestible protein per each kg gain compared to that required by the animals of Group II.

Table 3

Means, ± standard errors and standard deviations of the conformation scores, carcass scores, dressing percentage and fat percentage of the two groups of experimental animals

Traits	Group I. HF ♀×CHF ♂		Group II. HF♀×J♂	
	Mean ± S.E.	S.D.	Means ± S.E.	S.D.
Live animal conformation score*	40.37 ± 0.86	2.74	35.97 ± 0.78	2.47
Carcass conformation score**	42.05 ± 0.62	1.96	40.10 ± 0.79	2.51
Dressing percentage	60.25 ± 0.45	1.43	58.60 ± 0.55	1.75
Fat percentage***	6.67 ± 0.42	1.32	8.00 ± 0.62	1.97

* Live animal conformation score. Maximum 50 points distributed as follows: chest and neck 10; back and loin 15; ramp and round 15 and total appearance 10 points

** Carcass conformation score. Maximum 50 points distributed as follows: fore quarters 10; hind quarters 15; surface fat-, kidney fat-, marbling, 15 and total appearance 10 points

*** Fat percentage: includes the kidney fat and caul fat

The slaughter weight, hot carcass weight, offal and organ weight of the two groups of animals can also be found in Table 2. These data show that at the end of the experimental period and under the same nutritional conditions the animals of Group I were heavier than those of Group II regarding the relative slaughter weight and hot carcass weight. On the other hand, organ and offal weights were found to be heavier in Group II than in Group I. The means ± standard errors and standard deviations of the live animal conformation scores, carcass conformation scores, dressing percentage and fat percentage of the two cross-bred groups in this study are presented in Table 3. According to the results while the mean live animal conformation score of H. F. × C-H. F. (Group I) was 8.8 per cent greater than that of H. F. × J. (Group II), a slightly smaller difference of 3.9 per cent was found between the carcass conformation scores of the two groups.

The dressing percentage of Group I was found to be higher than that of Group II (60.3 per cent and 58.6 per cent respectively). On the other hand, the fat percentage of Group I was found to be lower (Table 3).

Simple correlation coefficients for relationship between live animal conformation scores, carcass conformation scores, dressing percentage and fat percentage within the two cross-bred groups are presented in Table 4. These data indicate the presence of a simple correlation coefficient between all the investigated traits. With the exception of the correlation between the live animal conformation score and dressing percentage of H. F. × J. (Group II) all the other correlations are statistically non-significant. The correlation between the live animal conformation score and dressing percentage within the animals of Group II was slightly significant (0.59, $P < 0.05$). It was also found that all the studied traits were positively correlated except the correlation between the dressing percentage and fat percentage of H. F. × J. (Group II) which was negatively correlated.

Table 4

*Simple correlation coefficients for relationships between live animal conformation scores, carcass conformation scores, dressing percentage and fat percentage within the two cross-bred groups**

Traits	Group I HF ♀×CHF ♂				Group II HF ♀×J ♂			
	1	2	3	Fat %	1	2	3	Fat %
1. Live animal conformation score	..	0.02	0.35	0.21	..	0.32	0.59	0.40
2. Carcass conformation score	..	..	0.19	0.08	..	..	0.52	0.46
3. Dressing percentage	..	..	..	0.33	..	..	..	—0.22

*Correlation at 0.55, $P < 0.05$

Discussion

The average daily gains were 1.171 and 0.994 kg for Hungarian Fleckvieh × Canadian Holstein Friesian (Group I) ; and Hungarian Fleckvieh × Jersey (Group II), respectively. A nearly similar result (0.994 kg daily gain) was obtained by HORN (1962) for the Hungarian Fleckvieh × Jersey and (1.141 kg daily gain) by KONCAR *et. al.* (1967) for the Yugoslav native Red and White × Jersey.

Using the Hungarian Fleckvieh × Ayrshire cross-bred bulls, SZUROMI (1969) found that the average daily gain was 0.973 kg, while it was 0.950 kg for the Hungarian Fleckvieh × Kostroma, as it was reported by NAGY (1965). Our results indicate that better daily gain could be reached by crossing the Hungarian Fleckvieh with Canadian Holstein Friesians, than that obtained by crossing Hungarian Fleckvieh with Jersey. However, the daily gain obtained by crossing the Hungarian Fleckvieh with Jersey was nearly similar to those reported by HORN (1962), NAGY (1965) and SZUROMI (1969).

The feed consumptions per one kg daily gain in the present study were 3.942 and 4.118 kg starch value, 697 and 731 g digestible protein for Group I and Group II, respectively. It could be concluded, that while greater daily gain was found with the animals of Group I than those of Group II, each kg daily gain required 4.30 per cent more starch value and 4.70 per cent more digestible protein in the animals of Group II than those of Group I. These results are in accordance with those reported by NAGY (1966).

The feed consumption per kg gain in the present study, as compared to those obtained, by other investigators with the crosses between Hungarian Fleckvieh and other dairy breeds, is satisfactory. Using the Hungarian Fleckvieh × Kostroma cross-bred bulls, the feed consumptions per kg gain were found to be 4.32 kg starch value and 693 g digestible protein (NAGY 1965), 3.64 kg starch value and 588 kg digestible protein (FARRAG 1971). The smaller figures reported by FARRAG (1971) were due to the intensive feeding and fattening system used during the whole experimental period.

The slaughter value in Table 3 shows that dressing percentage was 60.25 and 58.60 for H. F. × C-H. F. and H. F. × J., respectively. The difference between the two cross-breds was due to the heavier organs, offals and tallow of H. F. × J. than those of H. F. × C-H. F.

Since, in many countries slaughter cattle are purchased alive, the carcass quality of these live animals can be predicted by many trained evaluators with reasonable accuracy by evaluating the live animals' conformation (WHEAT—HOLLAND 1959; GOTTSCH *et al.* 1961, GREGORY *et al.* 1962, GREGORY *et al.* 1964, WILSON *et al.* 1964, VITLO—MAGEE 1965).

Using the Hungarian grading system Table 3 shows that the animals of H. F. × C-H. F. and H. F. × J. got 40.37 and 35.97 points for the conformation of the live animals and 42.05 and 40.10 points for the carcass conformation, respectively. While the Hungarian Fleckvieh breed is characterized as a good beef producer and of excellent fattening ability, the conformation of live animals of this breed and its crosses with Kostroma, as estimated by NAGY (1965), were found to be 39.75 and 39.33. The values for carcass conformation were 37.36 and 37.60 points, respectively, by using the same grading score, at about 509 kg slaughter weight.

Our results regarding the conformation of the live animals, in H. F. ×

C-H. F. and the carcass conformation of the two crosses are better than those obtained by the previous investigator.

The present results showed that the live animal conformation of H. F. × C-H. F. was better graded than those of H. F. × J., however, small differences were found concerning the carcass conformations of the two groups. These results support the conclusion of Gregory *et al.* (1962) and Horn—Dohy (1970) that the qualification differences between the slaughter animals must be based on the carcass conformation, rather than on the conformation of the live animals alone.

Simple correlation coefficients for relationships among the studied traits are shown in Table 4. These data indicate that positive correlations were found between all the investigated traits in the animals of the H. F. × C-H. F. Group I however, all these correlations are statistically non-significant.

On the other hand, the results regarding the correlation between examined traits in the animals of the H. F. × J. Group II indicate that only the dressing percentage was significantly correlated with the live animal conformation score (0.59, $P < 0.05$). Therefore, it seems, that the live animal conformation score can be used in predicting the dressing percentage of H. F. × J. cross-bred bulls.

Using the U. S. D. A. grading system, highly significant correlations were found between the dressing percentage both in live animal conformation by Lewis *et al.* (1969) and in carcass conformation by Kidwell *et al.* (1959).

While highly significant correlation was found between live animal score and carcass score (Kidwell *et al.* 1959, Gregory *et al.* 1962, Gregory *et al.* 1964, Lewis *et al.* 1969), the present experiment did not show significant correlation between the live animal conformation score and carcass conformation score either in the H. F. × C-H. F. or in the H. F. × J. groups, using the Hungarian grading system.

Finally the present results indicate that any procedure which assists in accurately predicting the carcass value of live-animals of Hungarian Fleckvieh × Canadian Holstein Friesian and Hungarian Fleckvieh × Jersey cross-bred bulls, would be of immense importance for both slaughtering and breeding.

Acknowledgements

I wish to express my deepest thanks to the staff members of the Department of Animal Husbandry, and to Dr. Gy. Pethes, Department of Physiology, University of Veterinary Science (Budapest). Thanks are also due to all the members and the workers at the Szentegát State Farm for providing the facilities and their kind help.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 27—34 (1976)

POLLEN PHYSIOLOGICAL STUDIES IN PEARS

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In the years of 1968, 1969 and 1970 the ability of developing pollen tube in vitro was studied in 6 diploid and 2 triploid pear varieties. The percentage pollen tube development showed a considerable fluctuation in each variety over the years examined. The quality of pollen depended on the process of reductive cell-division and on the climatic conditions. Annual differences in the percentage of pollen tube development were related to the extent of fruit set and the number of fertile seeds per fruit. On the basis of the percentage pollen tube development the varieties can be classified into three groups (bad = below 30 per cent, medium = 30—60 per cent, good = over 60 per cent)

Introduction

The pollen quality of pear varieties has been examined by a number of authors since the beginning of the century (Table 1). In Hungary pollen morphological and physiological studies in pears were performed by Nagy (1960). Continued studies were justified by the fact that results obtained under different ecological conditions and with different investigation methods varied to a great extent from variety to variety.

Owing to the above — first of all methodological — difficulties we examined the relationships between the temperature and the time of pollen tube development on one hand, and between the sugar concentration of the culture medium and the time of pollen tube development, on the other. It was on the basis of the results obtained that we started our present studies. The objectives of the studies were the following: 1. to study the annual trend of pollen tube development in the different varieties; 2. to determine the correlation between the annual trend of pollen tube development in vitro and the extent of fruit set obtained from controlled pollination; 3. to classify the varieties according to the extent of pollen tube development.

Material and Method

The pollen required for the investigations was obtained from trees of a variety collection on wild pear stocks planted in 1953 at the Érd–Elvira Station of the Horticultural Research Institute. Some 5—7 days before flowering bearing shoots were collected from all cardinal

Table 1

Percentage pollen tube development and somatic chromosome numbers of some

Variety	CHOLLET (1965)	FLORIN (1927)	KAMLAH (1928)	KOBEL (1927)	KOBEL (1954)	MIEDZYRZECKI (1933–34)
"Bosc kobakja "			71—74			
"Clapp kedveltje"		30—70	85—91			55
"Diel vaj"	14	0.0—30	15—16	6	6	5
"Esperen bergamottja"						
"Hardenpont téli vaj"			88—93		31	
"Hardy vaj"	69			72	72	
"Nemes Krasszán"	45					
"Pap körte"	5	0.0—30		4	4	
"Serres Olivér"						40
"Téli esperes"						
"Vilmos körte"	64	30—70	67—73	46	46	

points of the mid-zone of the crown and made to flower at 20 °C under laboratory conditions. The average pollen samples were taken from fruit spurs, from lateral flowers third from the base of the cluster. Examinations on the pollen tube development were performed according to NYÉKI (1971); saccharose concentration was 15 per cent, germination was carried on over 160 minutes in a thermostat of 23—25 °C temperature. Three drops per variety were examined; percentage pollen tube formation was determined on the basis of 2×50 pollens in each drop. Pollens with tubes longer than the pollen diameter were considered as germinated.

Results

1. *Pollen tube development trends per variety and year.* Percentage pollen tube formation in the different varieties showed considerable annual fluctuations due to annual changes in the quality of pollen (Table 2).

In the varieties examined the quality of pollen depended on the process of reductive cell-division and on climatic factors — first of all temperature and precipitation (NYÉKI 1970).

The extent of pollen tube formation can be influenced by many factors.

important pear varieties according to literary data

Moffet (1934)	Rudloff—Schanderl (1950)	Schanderl (1932)	Schanderl (1934)	Uhlik (1964)	Somatic chromosome number (n = 17)	References
59	30—100	95—98	33—100	78.4	34	Moffet (1934) Uhlik (1964)
	30—100	95—99	33—100	83.8	34	Moffet (1934) Uhlik (1964)
12	0.0—30	10—15	0.0—33	23.3	45 (?)	Kobel (1927) ? Miedzyrzecki (1933—34) Moffet (1934) Uhlik (1964)
	30—100		33—100	50.4	34	Uhlik (1964)
	30—100		33—100		34	Kobel (1954)
82	30—100		33—100	74.7	34	Kobel (1927) Moffet (1934) Uhlik (1964)
		20—25			34	Chollet (1965)
	0.0—30	30	0.0—33	6.1	55 (?)	Kobel (1927) ? Uhlik (1964) Chollet (1964)
	30—100	70—80	0.0—33		34	Miedzyrzecki (1933—34)
	30—100		33—100	75.4	34	Uhlik (1964)
56	30—100	90—95	33—100	53.0	34	Kobel (1927) Moffet (1934) Uhlik (1964)

Table 2

Trends in the pollen tube development percentages of some important pear varieties in the years of the investigations

Variety	Years of investigations			Mean	Characterization of the tube forming capacity
	1968	1969	1970		
1. "Bosc kobakja"	42.5	68.3	57.8	56.2	medium
2. "Clapp kedveltje"	58.2	91.7	78.2	76.0	good
3. "Diel vajkörte"	3.1	8.7	5.0	5.6	bad
4. "Hardenpont téli vaj"	39.0	53.1	40.5	44.2	medium
5. "Nemes Krasszán"	23.6	50.3	39.5	37.8	medium
6. "Pap körte"	3.8	6.9	4.3	5.0	bad
7. "Serres Olivér"	28.4	36.1	30.7	31.7	medium
8. "Vilmos körte"	68.3	72.6	71.0	70.6	good

For example, according to the investigations of Sandsten cit. Gardner *et al.* (1952) pollens originating from old apple trees developed tubes in 39.8 per cent, while those taken from young trees in 56.5 per cent. The differences suggest — in other authors' opinion as well — nutrition — condition correlations, which may manifest themselves in the capacity of pollen tube formation too.

The different methods of sample taking may also result in very great differences. In the apple variety Lanés Prince Albert VISSER (1955) found differences in the percentage of pollen tube development according to the sections of the inflorescence the flowers had been taken from. There are also data available on differences in the pollen tube developing percentages of pollens of various size. In the course of pollen physiological studies performed in pear varieties NAGY (1960) found that in a sugar solution of 15 per cent concentration it was the small pollens, while in a 10 per cent solution the large pollens that germinated better.

The extent of pollen tube formation in the various pear varieties depends primarily on the ploidy level (KOBEL 1927, MOFFET 1934 etc.). In triploid varieties forming irregular tetrades the percentage of pollen tube development is very low. But even in the case of triploid varieties differences between the years can be observed. In favourable seasons certain triploid pear varieties may attain a tube development percentage which is close to the values attained by certain diploid varieties in unfavourable years (UHLIK 1964, CHOLLET 1965).

2. *Relationship between the annual percentage of pollen tube development in vitro and the extent of fruit set.* The annual change in the percentage of pollen tube development (Table 2) can also be related with the results of fruit set studies performed in the field.

We use three diploid and one triploid varieties as models to demonstrate annual changes in the extent of fruit set (Tables 3 and 4). The value of pollen tube formation in vitro, the number of ripe fruits and the average number of fertile seeds per fruit were lower in 1968 than in 1969. As pointed out earlier (NYÉKI 1970) the low 1968 values of pollen tube formation and fruit set can be attributed partly to the dry hot weather prevailing at the time of fruit but differentiation and low temperatures during the meiosis, partly to low temperatures at the time of flowering and pollination. Fruit set found in the triploid pear varieties as well as the low number of germinative seeds were the results of a pollen sterility caused by cytological conditions — lack of balance between gametes and embryo — and were not caused by incompatibility (CRANE—LEWIS 1942, KOBEL 1954).

3. *Classification of pear varieties by the extent of pollen tube formation.* According to the extent of pollen tube development the varieties were divided into three groups (Table 2):

a) low pollen tube developing capacity = tube formation below 30 per cent;

b) medium = between 30 and 60 per cent;

c) good = over 60 per cent.

Table 3

Fruit setting- and pollinating capacities expressed in the percentage of ripe fruits; average number of germinative seeds per fruit in pear varieties
(1968—1969, Érd-Elvira)

Difference		Pollinating capacity (when used as pollen variety)		Difference	
Percentage of ripe fruits	Percentage of germinative seeds per fruit	1968	1969	Percentage of ripe fruits	Percentage of germ. seeds per fruit
+10.0	+2.6	86 : 3.2 : 2.0	96 : 12.5 : 4.3	+9.3	+2.3
—	—	126 : 4.8 : 4.9	71 : 9.9 : 4.8	+5.1	—0.1
+7.3	+5.0	86 : 6.9 : 8.0	94 : 10.6 : 4.6	+2.7	—3.4
+6.6	+3.9	81 : 2.5 : 3.5	118 : 10.2 : 3.0	+7.7	—0.5
+20.0	+0.6	116 : 8.6 : 7.4	85 : 2.3 : 8.0	—6.3	+0.6
—	—	—	—	—	—
—	—	—	128 : 1.5 : 1.0	—	—
+11.5	+5.7	196 : 0.0 : 0.0	87 : 5.7 : 2.7	+5.7	+2.7
+25.8	—4.6	88 : 0.0 : 0.0	110 : 18.2 : 2.4	+18.2	+2.4
+9.3	+2.3	100 : 0.0 : 0.0	174 : 10.0 : 2.6	+10.0	+2.6
—	—	88 : 0.0 : 0.0	86 : 6.9 : 3.7	+6.9	+3.7
+34.0	+7.1	160 : 1.3 : 7.0	106 : 9.4 : 5.8	+8.1	—1.2
—	—	122 : 0.0 : 0.0	109 : 15.6 : 5.6	+15.6	+5.6
+30.4	+8.1	70 : 10.0 : 6.0	79 : 6.3 : 6.5	—3.3	+0.5
—	—	92 : 0.0 : 0.0	126 : 11.9 : 4.1	+11.9	+4.1
—	—	—	124 : 16.1 : 0.6	—	—
—	—	—	102 : 0.0 : 0.0	—	—
—4.1	+3.3	74 : 1.3 : 1.0	—	—	—
+5.2	+1.0	102 : 1.9 : 4.0	—	—	—
+5.9	+2.0	108 : 0.0 : 0.0	—	—	—
+7.7	—0.5	84 : 3.5 - 2.0	245 : 10.1 : 5.9	+6.6	+3.9
+15.6	+5.6	—	72 : 12.5 : 7.8	—	—
+16.9	+6.3	104 : 0.0 : 0.0	98 : 18.7 : 5.4	+18.4	+5.4
+4.5	+0.3	76 : 11.8 : 5.2	100 : 5.0 : 3.8	—6.8	—1.4
—	—	116 : 3.5 : 4.0	108 : 3.9 : 6.5	+0.4	+2.5
—	—	—	—	—	—
+4.6	±0.0	136 : 1.2 : 1.5	—	—	—
+11.3	+2.0	—	120 : 3.3 : 0.0	—	—
+9.5	+1.0	216 : 0.0 : 0.0	120 : 10.0 : 0.0	+10.0	±0.0
±0.0	±0.0	130 : 0.0 : 0.0	84 : 16.7 : 0.0	+16.7	±0.0
—	—	—	132 : 3.0 : 1.0	—	—

Variety (♀)	Partner varieties (♂)	Fruit setting capacity (when used as seed variety)	
		1968	1969
"Clapp kedveltje"	"Vilmos körte"	100 : 0.0 : 0.0*	174 : 10.0 : 2.6*
	"Bosc kobakja"	—	70 : 4.3 : 8.8
	"Pringalle vaj"	82 : 4.9 : 2.7	82 : 12.2 : 7.7
	"Hardenpont"	84 : 3.5 : 2.0	245 : 10.1 : 5.9
	"Dupuit asszony"	118 : 3.2 : 6.0	112 : 23.2 : 6.6
"Clapp kedveltje"	"Pap körte"	—	122 : 1.6 : 0.0
	"Diel vaj"	—	134 : 0.0 : 0.0
"Vilmos körte"	"Serres Olivér"	172 : 0.0 : 0.0	104 : 11.5 : 5.7
	"Nemes Krasszán"	102 : 0.0 : 0.0	128 : 25.8 : 4.6
	"Clapp kedveltje"	86 : 3.2 : 2.0	56 : 12.5 : 4.3
	"Bosc kobakja"	—	231 : 16.9 : 7.3
	"Pringalle vaj"	104 : 0.0 : 0.0	106 : 34.0 : 7.1
	"Hardenpont téli vaj"	—	72 : 12.5 : 7.8
	"Dupuit asszony"	73 : 0.0 : 0.0	92 : 30.4 : 8.1
	"Téli esperes"	—	102 : 0.0 : 0.0
	"Pap körte"	—	74 : 9.5 : 1.0
	"Diel vaj"	—	64 : 0.0 : 0.0
"Hardenpont téli vaj"	"Téli esperes"	100 : 6.0 : 4.7	104 : 1.9 : 8.0
	"Serres Olivér"	224 : 1.8 : 4.0	71 : 7.0 : 5.0
	"Nemes Krasszán"	95 : 0.0 : 0.0	102 : 5.9 : 2.0
	"Clapp kedveltje"	81 : 2.5 : 3.5	118 : 10.2 : 3.0
	"Vilmos körte"	122 : 0.0 : 0.0	109 : 15.6 : 5.6
	"Pringalle"	102 : 0.0 : 0.0	71 : 16.9 : 6.3
	"Dupuit asszony"	110 : 5.5 : 6.5	100 : 10.0 : 6.8
	"Bosc kobakja"	—	175 : 15.6 : 5.4
	"Pap körte"	—	98 : 6.1 : 0.3
	"Diel vaj"	73 : 0.0 : 0.0	86 : 4.6 : 0.0
"Diel vaj"	"Téli esperes"	122 : 0.0 : 0.0	88 : 11.3 : 2.0
	"Serres Olivér"	152 : 3.7 : 1.0	106 : 13.2 : 2.0
	"Nemes Krasszán"	120 : 0.0 : 0.0	102 : 0.0 : 0.0
	"Pap körte"	—	90 : 0.0 : 0.0

Note: * = the first group of figures means the number of flowers treated, the second group, after the first colon, the percentage of ripe fruits and the last group of figures, after the second colon, means the average number of germinative seeds per fruit

Table 4

Changes in the fruit setting- and pollinating capacities of pear varieties in the case of partner varieties with different somatic chromosomes

Variety (♀)	Partner varieties (n = 17) (♂)	Fruit setting capacity (when used as seed var.)		Difference		Pollinating capacity (when used as pollen variety)		Difference	
		1968	1969	Percent of ripe fruits	Percent of germinative seeds per fruits	1968	1969	% of ripe fruits	% of germinative seeds per fruit
"Clapp kedveltje"	2 n	2.9 : 2.7*	11.9 : 6.3*	+9.0	+3.6	5.2 : 5.2	9.1 : 4.9	+3.9	—0.3
	3 n	—	0.8 : 0.0	—	—	—	1.5 : 1.0	—	—
	Varietal mean	2.9 : 2.7	8.8 : 4.5	+5.9	+1.8	5.2 : 5.2	7.8 : 4.3	+2.6	—0.9
"Vilmos körte"	2 n	0.6 : 0.4	16.7 : 5.6	+16.1	+5.2	1.4 : 1.6	10.5 : 4.2	+9.1	+2.6
	3 n	—	4.8 : 0.5	—	—	—	8.1 : 0.3	—	—
	Varietal mean	0.6 : 0.4	14.3 : 4.6	+13.7	+4.2	1.4 : 1.6	10.0 : 3.4	+8.6	+1.8
"Hardenpont téli vaj"	2 n	2.3 : 2.7	10.5 : 5.2	+8.2	+2.5	3.1 : 2.3	9.9 : 5.9	+6.8	+3.6
	3 n	0.0 : 0.0	5.3 : 0.1	+5.3	+0.1	1.2 : 1.5	—	—	—
	Varietal mean	1.9 : 2.3	9.5 : 4.2	+7.6	+1.9	2.9 : 2.2	9.9 : 5.9	+7.0	+3.7
"Diel vajkörte"	2 n	1.2 : 0.3	8.1 : 1.3	+6.9	+1.0	0.0 : 0.0	10.0 : 0.0	+10.0	±0.0
	3 n	—	0.0 : 0.0	—	—	—	3.0 : 1.0	—	—
	Varietal mean	1.2 : 0.3	6.1 : 1.0	+4.9	+0.7	0.0 : 0.0	3.2 : 0.2	+3.2	+0.2

Note: * = the first group of figures represents the percentage proportion of ripe fruits to flowers treated; the second group, after the colon, the average number of germinative seeds per fruit

Conclusions

Annual fluctuations in the extent of pollen tube development are attributed to meiotic abnormalities. Varieties with high tube formation percentage may show a low percentage of fruit set, and vice versa, those with medium tube formation percentage may give satisfactory pollination results — depending on the sexual compatibility of the variety and temperatures prevailing at the time of pollination. Varieties with good quality pollen (high degree of fertility) and high tube forming capacity should be preferred as pollen partners. The triploid varieties cannot be recommended as pollen donors owing to their low capacity to develop pollen tubes and low pollinating percentage.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 35—43 (1976)

DETERMINATION OF THE NUTRIENT VALUE OF FEEDS BY PARTIAL ANALYSIS, ON THE BASIS OF THE RELATIONSHIP OF THEIR CHEMICAL COMPOSITION

By

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On samples of 219 maize silage, 570 alfalfa hay, 262 corn meal, 120 wheat bran and 87 extrahated sunflower meal the authors examined the relationship between the crude composition of feeds and digestible crude protein and starch equivalent calculated by traditional methods on the basis of complete analysis. According to the authors' conclusion the relationship of the chemical composition of feeds may give a theoretical basis for a calculation system which allows the determination of the nutrient value of feeds within given error limits on the basis of partial analysis with the use of the regression equations reported in the paper.

Introduction

Feeding specialists have long been dealing with the feeding value of feedstuffs determined by Weende full analysis and with the relationships of the chemical composition of given feeds with their starch equivalent and digestible crude protein content, respectively. While researchers make every effort on the one hand to improve the parameters to determine the productive value of feeds by developing much more exact, but more labour-consuming procedures, there are demands arising on the other hand for simple and prompt analytical and measuring methods to determine the feeding value of feeds.

Many reports are dealing with the determination of the feeding value of feeds based on partial analysis.

Schneeberger—Schoch (1968) carried out analyses of 20 different samples of maize silage. From the high correlation the conclusion was to be drawn that the starch equivalent of maize silage might be determined both from the original dry matter and crude fibre content related to the absolute dry matter content, with the help of the regression equations:

1. Starch equivalent (dry matter 100 per cent) = 84.094 — 1.33 crude fibre (100 per cent dry matter)

$$r = -0.919 \qquad s = \pm 2.410$$

2. Starch equivalent (original dry matter) = 3.587 + 0.732 dry matter

$$r = 0.978 \qquad s = \pm 0.942$$

The latter method is more accurate.

The digestible crude protein content of maize silage related to absolute dry matter content is calculated by the regression equations:

1. Digestible crude protein (dry matter 100 per cent) = —3.631 + + 0.987 crude protein (dry matter 100 per cent)

$$r = 0.887 \qquad s = \pm 0.502$$

2. Digestible crude protein (dry matter 100 per cent) = —3.360 + + 1.159 pepsin hydrochloric digestible protein (dry matter 100 per cent)

$$r = 0.983 \qquad s = \pm 0.197$$

On the basis of MSZ 6830-66 standard (Hungary) the digestible crude protein content and starch equivalent of industrial feedstuffs and farmgrown feeds may be calculated also from the nutrient which is mostly influenced by the nutritive value. Thus, instead of the full analysis, it is sufficient to have a few analytical data (partial analysis) to estimate the feeding value. By this way of calculation the same results may be gained, as by the full analysis (stated by Kellner).

In the case of corn meal, e.g.:

Starch equivalent = dry matter × 0.92.

The results must be given with an accuracy of 10 grams.

BAINTNER (1967) constructed a tabulation, on the basis of which the starch equivalent and digestible crude protein content of feeds may be calculated directly from the chemical composition.

The Bedrijfslaboratorium woor Groond- en Gewasonderzoek in the Netherlands (1965) reported that the determination of feeding value by Weende-analysis made on the basis of a table developed there, has a proper accuracy for practical use and shows a high correlation with it. Based on the analysis of 955 samples, moreover on the correlation and regression analyses it was to be stated that the starch equivalent of feeds was influenced first of all by the crude fibre and crude ash content (KNAUER 1971).

NEHRING (1966a, 1966b) found the correlation between crude protein content and digestible protein content of feeds to be practically $r = +1.0$.

NAYDENOV (1967) calculated the digestible protein content (y) from the crude protein content (x) referred to the dry matter content after the following relationships:

1. y (green fodder) $= 0.922\,x - 2.87$ $(r = 0.973)$
2. y (hay) $= 0.915\,x - 3.70$ $(r = 0.973)$
3. y (silage) $= 0.896\,x - 3.15$ $(r = 0.985)$

Holther—Reid (1959) estimated the digestible crude protein content of alfalfa hay as follows:

$$y = 0.867\,x - 2.56 \qquad r = 0.995 \qquad s = \pm 0.42$$

where

y = digestible crude protein in per cent of dry matter
x = crude protein in per cent of dry matter.

Beyer—Hoffmann (1971) studied the relationships between the crude protein (x) and digestible crude protein in green fodders. The results of these studies are as follows:

$$y\ (\text{alfalfa, first cutting}) = -3.37 + 0.99x \pm 0.40$$
$$r = +0.996 \qquad (n = 71)$$

$$y\ (\text{red clover}) = 91.7 - 1.35x \pm 2.6$$
$$r = -0.937 \qquad (n = 102)$$

They suggested that certain chemical analysis data are of remarkable importance in definite feed varieties. Dijkstra (1954) worked out some regression equations for fresh grass, artificially dehydrated grass, grass silage and hay. He calculated the digestible crude protein content of hay related to the absolute dry matter content (d) on the basis of crude ash content (m) and of crude protein content (x), and the starch equivalent related to the absolute dry matter content (s) on the basis of crude fibre (y) and crude ash (m) content expressed in per cent of dry matter:

$$d = 0.771(x - 13) + 0.030(m - 9) + 7.265$$

$$s = -1.666(y - 32) - 1.004(m - 9) + 38.06$$

In his latest report Dijkstra (1968) has emphasized that the crude protein and digestible crude protein have high correlations with crude fibre and starch equivalent in grass hay and in fresh grass, but also in other feeds.

For the determination of the real productive value of feeds he considers the starch equivalent and digestible crude protein determined by chemical analysis to be sufficient.

Material and Method

Five kinds of feeds (maize silage, alfalfa hay, corn meal, wheat bran, extracted sunflower meal) playing the greatest role in practical animal nutrition in Hungary were chosen for the investigation, which comprised of samples analyzed according to the MSZ-6830-66 (Hungarian Standard) in the Central Laboratory of the Research Institute for Animal Husbandry within the period of 1964—1970. Both bulky and concentrated feeds were included.

Within the course of data processing* all the samples involved in the calculations underwent a selection by a computer. That is, samples having parameters diverging either in a positive or a negative direction from the average with a more than threefold value of the standard error were omitted from the calculation. The aim of this selection was not to diminish the accuracy of the correlation coefficients and regression equations by the non-characteristic values of the given feeds. To avoid the interfering effects caused by the different dry matter contents, the estimations were made with the values related to the absolute dry matter content. The variables used in our investigations:

x_1 = dry matter
x_2 = crude ash
x_3 = organic matter
x_4 = crude protein
x_5 = crude fat
x_6 = crude fibre
x_7 = N-free extracts
z = digestible crude protein
y_0 = starch equivalent calculated by conventional methods
$y_{1 \cdots 4}$ = starch equivalent calculated by multiple regression equations
$* = P\% < 5$; $** = P\% < 1$; $*** = P\% < 0.1$.

Results

Single and multiple regression and correlation between the chemical composition and nutritive value of feeds were analyzed. Several workers (Holther—Reid 1959, Nehring 1966a, 1966b, Naydenov 1967, Schneeberger—Schoch 1968, Beyer—Hoffmann 1971) analyzed the relationships between the digestible crude protein content and crude protein content of feeds. The correlation coefficients and regression equations established in our work are shown in Table 1. From the regression equations the conclusion may be drawn that the digestible crude protein content per unit of crude protein content in the different feeds analyzed is — because of the different digestibility of the crude protein — the greater, the better is the digestibility. We take it for granted to calculate the digestible crude protein content of feeds conventionally, directly from the crude protein content, although Beyer—Hoffmann (1971) suggest the application of regression equations for this, too. The situation is quite different in the case of the starch equivalent. Considering the insufficient laboratory capacity and the small number of samples analyzed, it seemed to be reasonable to search for a method by which the starch equivalent of feeds fed in the largest quantity in Hungary may be determined not only by the whole, but also by partial analysis. It is an essential requirement for the index of feeding value to give accurate information about the productive value of the feed analyzed. The crude nutrients estimated in our paper and their relationships with the feeding value of different feeds may constitute a theoretical basis for a calculation system based on partial analysis, that is able to satisfy the above mentioned demands.

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* The data were processed by electronic computer of MAVEMI (Engineering Bureau of the Hungarian Association of Chemical Industry).

Table 1

Regression equations calculated for crude protein content of feeds (x_1) and digestible crude protein (z) related to absolute dry matter content

Feeds	n	Regression equation	Correlation coefficient (r)	Standard error %
Maize silage	219	$z = 0.05 + 0.55x_4$	0.895***	10.45
Alfalfa hay	570	$z = 1.39 + 0,64x_4$	0.933***	6.18
Corn meal	262	$z = 0.07 + 0.82x_4$	0.984***	1.76
Wheat bran	120	$z = 0.35 + 0.81x_4$	0.990***	1.01
Extr. sunflower meal	87	$z = -1.12 + 0.91x_4$	0.916***	4.78

Table 2

Estimates of the starch equivalent of feeds y_{1-4} by regression equations

Feeds	n	Regression equation	Multiple correlation coefficient (r)	Standard error %
1.	2.	3.	4.	5.
Maize silage	219	$y_1 = 74.98 - 0.7x_6$	0.590***	6.54
		$y_2 = 78.00 - 0.32x_4 - 0.70x_6$	0.606***	6.45
		$y_3 = 69.89 - 0.13x_4 + 0.95x_5 - 0.64x_6$	0.744***	5.42
		$y_3' = 82.00 - 0.15x_4 - 0.64x_6 - 0.82x_2$	0.733***	5.51
		$y_4 = 74.24 - 0.01x_4 + 0.89x_5 - 0.59x_6 - 0.74x_2$	0.830***	4.52
Alfalfa hay	570	$y_1 = 57.84 - 0.68x_6$	0.836***	7.00
		$y_2 = 55.47 + 0.08x_4 - 0.66x_6$	0.838***	6.98
		$y_3 = 54.93 + 0.07x_4 + 0.30x_5 - 0.66x_6$	0.839***	6.95
		$y_3' = 62,39 + 0.16x_4 - 0.75x_6 - 0.59x_2$	0.876***	6.19
		$y_4 = 62.04 + 0.15x_4 + 0.16x_5 - 0.75x_6 - 0.58x_2$	0.875***	6.18
Corn meal	262	$y_1 = 87.40 + 1.02x_5$	0.624***	0.89
		$y_2 = 89.38 - 0.22x_4 + 1.05x_5$	0.656***	0.86
		$y_3 = 90.42 - 0.23x_4 + 1.06x_5 - 0.40x_6$	0.702***	0.81
		$y_{3'} = 92.05 - 0.22x_4 + 0.87x_5 - 0.96x_2$	0.768***	0.73
		$y_4 = 92.46 - 0.23x_4 + 0.89x_5 - 0.26x_6 - 0.86x_2$	0.784***	0.71
Wheat bran	120	$y_1 = 58.89 - 0.33x_6$	0.215*	3.72
		$y_2 = 57.12 + 0.11x_4 - 0.34x_6$	0.223*	3.71
		$y_3 = 56.83 + 0.02x_4 + 0.55x_5 - 0.43x_6$	0.281**	3.65
		$y_4 = 56.40 + 0.10x_4 + 0.56x_5 - 0.38x_6 - 0.25x_2$	0.296**	3.63
Extr. sunflower meal	87	$y_1 = 38.22 + 0.49x_4$	0.597***	4.93
		$y_2 = 45.60 + 0.40x_4 - 0.22x_6$	0.637***	4.74
		$y_3 = 40.42 + 0.47x_4 + 0.79x_5 - 0.17x_6$	0.652***	4.66
		$y_3' = 35.83 + 0.57x_4 + 1.31x_5 - 0.40x_2$	0.643***	4.70
		$y_4 = 42.11 + 0.48x_4 + 0.99x_5 - 0.16x_6 - 0.35x_2$	0.660***	4.62

The bivariate and multiple regression equations for the determination of the starch equivalent estimated on the basis of the chemical composition related to the absolute dry matter content are shown in Table 2. In these equations the starch equivalent figures are the dependent variables and the nutrient composition figures are the independent variables, depending on the number of variables. The table includes also the multiple correlation coefficient and the standard errors of the regression equations. The starch equivalent in maize silage and alfalfa hay was highly influenced by the crude fibre content related to the absolute dry matter content. When the crude protein content was involved as independent variable — as it was to be expected —, it did not improve the accuracy of the information in any of the feeds analyzed (three-variate regression equations). With regard to the fact that in any case the crude protein must be determined also by partial analysis, we thought it to be reasonable to include the crude protein contents into the equations. It may be stated that the difference between the accuracy of the four-variate and five-variate equations based on the full analysis may be also neglected. In our opinion, in the case of both bulky feeds it is most important to determine the crude fibre content by laboratory analysis besides the dry matter content estimation. This concerns also the wheat bran. The primary factor for starch equivalent is the crude fat in corn meal and the crude protein in the extracted sunflower. If the calculations are made with four-variate regression equations based on partial analysis the determination of the crude fat content in maize silage and alfalfa hay may be neglected. Thus, the extracting capacity will be released for the determination of the fat content in feeds, where e.g. in maize, the starch equivalent will be greatly influenced by the crude fat content. Therefore, in the case of corn meal, the analysis of the crude fibre content may be neglected. When wheat bran and extracted sunflower are analyzed by using the four-variate regression equation, the crude ash may be left out. The five-variate regression equations can make easier only the conventional arduous ways of calculation based on full analysis. Their publication seemed to be reasonable for comparing with two-, three-, and four-variate regression equations and multiple correlation coefficients and standard errors. It depends on the number of samples analyzed, the capacity of the laboratory and the time available, which nutrients will be chosen. The dry matter content and digestible crude protein content of feeds must always be determined. The next nutrient of feeds, except maize, is the crude fibre, that must be inevitably determined. This is followed by the analysis of crude fat in corn meal, maize silage, wheat bran and extracted sunflower meal, and the analysis of crude ash in alfalfa hay and corn meal.

Some efforts may be found in the literature to determine the starch equivalent by regression equations based on the original dry matter content and SCHNEEBERGER—SCHOCH (1968) furthermore GROSS (1972) even calculated the starch equivalent of maize silage directly from the dry matter content. The two- and three-variate regression equations calculated in this way are summarized in Table 3. By comparing the 2nd and 3rd tables, it may be stated, however, that the estimation of the starch equivalent solely on the basis of the dry matter content is much more inaccurate, or it is not possible at all (alfalfa hay). The estimate of three-variate regression equations gives less reliable information, than that of the equations determined on the basis of absolute dry matter content. The crude protein related to the original dry matter content must always be determined, and does not increase the informative value of estimates as independent variable in the equation. As our investigations proved the estimate for absolute dry matter content is much more suitable for this. The way of estimation will be demonstrated by an example of corn meal, that is not included in our material.

Table 3

Estimates of starch equivalent of feeds (y_{1-2}) based on original dry matter content by bivariate and multiple regression equations

Feeds	n	Regression equation	Multiple correlation coefficient r	Standard error %
Maize silage	219	$y_1 = 0.46 + 0.55x_1$	0.921***	8.77
		$y_2 = 1.10 + 0.66x_1 - 0.52x_6$	0.937***	7.90
Alfalfa hay	570	$y_2 = -2.60 + 0.61x_1 - 0.68x_6$	0.881***	6.84
Corn meal	262	$y_1 = 1.35 + 0.91x_1$	0.897***	1.69
Wheat bran	120	$y_1 = -9.10 + 0.66x_1$	0.564***	3.84
Extr. sunflower meal	87	$y_1 = 34.25 + 0.50x_4$	0.602***	5.18

Sample: corn meal 23/815	23. 3. 1971	
Chemical composition:	per cent	per cent
Original dry matter	91.2	$x_1 = 100.00$
Crude protein	8.6	$x_4 = 9.43$
Crude fat	4.6	$x_5 = 5.04$
Crude fibre	2.3	$x_6 = 2.52$
Crude ash	1.4	$x_2 = 1.54$
N-free extracts	74.3	$x_7 = 81.47$

Feeding value calculated from full analysis according to the standard:	per cent	per cent
Starch equivalent	84.7	$y_0 = 92.87$
Digestible crude protein	7.1	$z = 7.78$

The course of estimates based on absolute dry matter content:

1. With bivariate regression equation

$y_1 = 87.40 + (1.02 \cdot 5.04) = 92.54$ per cent
$92.54 \cdot 91.2 = 84.5$ starch equivalent per cent
deviation —0.2 per cent

2. With three-variate regression equation

$y_2 = 89.38 + (—0.22 \cdot 9.43) + (1.05 \cdot 5.04) = 92.60$ per cent
$92.60 \cdot 91.2 = 84.5$ starch equivalent per cent
deviation —0.2 per cent

3. With four-variate regression equation

$y_3 = 92.42 = (—0.23 \cdot 9.43) + (1.06 \cdot 5.04) + (—0.40 \cdot 2.52) = 92.58$ per cent
$92.58 \cdot 91.2 = 84.5$ starch equivalent per cent
deviation —0.2 per cent

$y'_3 = 92.05 + (—0.22 \cdot 9.43) + (0.87 \cdot 5.04) + (—0.96 \cdot 1.54) = 92.88$ per cent
$92.88 \cdot 91.2 = 84.8$ starch equivalent per cent
deviation +0.1 per cent

4. With five-variate regression equation

$y_4 = 92.46 + (—0.23 \cdot 9.43) + (0.89 \cdot 5.04) + (—0.26 \cdot 2.52) + + (—0.86 \cdot 1.54) = 92.81$ per cent
$92.81 \cdot 91.2 = 84.7$ starch equivalent per cent
deviation 0.0

Estimate on the basis of the original dry matter:

$y_1 = 1.35 + (0.91 \cdot 91.2) = 84.3$ starch equivalent per cent
deviation —0.4 per cent

Conclusions

On the basis of the regression equations calculated, a good possibility presents itself to estimate the starch equivalent of feed analyzed by partial analysis with the accuracy reported in this paper.

The kind of equations that may be used in a given case depends on the demands made for the accuracy of estimating the feeding value, on the number of samples analyzed, on the laboratory capacity available, on the "time" factor. These factors must be considered to decide the number of variables in the regression equations. Finally these determine which of the nutrients of a given feed must be included in the determination by partial analysis.

Generally, the estimates based on absolute dry matter are more accurate than that of the original dry matter, or by the calculation based on this.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 45—50 (1976)

EFFECTS OF CCC AND CHOLINE UPON SHOOT GROWTH AND TOTAL CARBO-HYDRATE AND CRUDE PROTEIN CONTENT IN 2-YEAR-OLD WILD PEAR SEEDLINGS

By

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CCC and choline treatments of 0, 500, 1000, 2000 and 4000 ppm concentrations were applied to two year old pear seedlings on 6th and 20th May. Between CCC and choline treated plants differences were shown in plant height, root-collar diameter and average length of internode as well as in the total carbo hydrate and crude protein content of the shoot and root. On the basis of four treatments per chemical there was a significant increase in the total carbo hydrate content of the shoot and the crude protein content of the root. The author has arrived at the conclusion that the shortening of the juvenile habit stage of wild pear with CCC is a problem much more difficult to solve than was expected on the basis of investigations on 1 year old seedlings.

Introduction

The reduction of shoot growth by CCC was the most efficient in wheat and pear. The researchers see the perspectives of the chemical in its increasing the yield and maintaining the bearing stage of bearing trees as well as promoting the non-bearing trees in turning into bearing (Modlibowska 1965, Marcelle—Raskin 1967, Fiorino—Loretti 1968, Dennis 1968, Brian 1970, Wertheim 1972).

Our investigations have been built on seedlings from the beginning, since our aim is to shorten the juvenile habit stage of wild pear turning very late into bearing, and finding the possible ways and means of it, respectively. Beyond the functional morphological characters we tried to examine the phenomenon first of all on the basis of the parameters of the woody parts (Surányi 1970a, b).

According to our observations the one year old seedlings do not overcome the inhibiting effect of CCC in the year of the treatment, and at a concentration over 2000 ppm growth retardation is maintained in the next year too. What will happen, on the other hand, if we treat two year old, strengthened and unpruned seedlings with CCC? We consider the investigation of this question and the comparison of the results with those obtained for year old plants important; moreover, in the present paper we give an account of investigations concerning the basic compound — choline — too.

Material and Method

In the autumn of the year preceding the treatment one year old wild pear seedlings were transplanted to culture beds, then on 6th and 20th May treated with 2 × 250 ml CCC and choline per plant at concentrations of 0, 500, 1000, 2000 and 4000 ppm. Of the morphological surveys of two year old seedlings only the results obtained at the end of the vegetation are presented (Table 1), namely the data of the root-collar diameter, the plant height and the (calculated) average length of internodes.

The number of replications was 2 × 10; the above-ground and root parts of defoliated seedlings were separated, and in the homogenisates prepared from them the total carbohydrate and crude protein contents determined with the methods described in an earlier paper; evaluation was carried out on the basis of 3 sample groups (SURÁNYI 1972). The morphological results and those relative to the components were statistically evaluated.

Results

Choline and CCC had different effects on the pear seedlings; according to the total plant height the choline treatments stimulated, while the CCC treatments (with the exception of 500 ppm) inhibited the shoot growth. Plant height was in very close correlation with the diameter of the root-collar ($r = +0.454$ and $p = 0.1$ per cent). The average length of the internode was

Table 1

Habit response of 2-year-old wild pears to treatments with choline and CCC

Treatment ppm	Collar diameter mm	Height of plant cm	Length of internode cm	Number of internodes pc
Control	56.1	46.9	1.38	33.9
Choline				
500	70.4*	69.6***	1.36	
1000	61.6	62.8**	1.25	
2000	70.9**	68.0***	1.29	50.6
4000	65.2	59.0*	1.23*	
CCC				
500	73.1**	63.3**	1.30	
1000	55.0	31.3**	0.86***	
2000	50.6	31.2**	0.75***	41.7
4000	60.0	28.0***	0.76***	
L.S.D. 1%	14.6	12.6	0.19	—

* p = 5%
** p = 1%
*** p = 0.1%

slightly decreased by choline and very intensively by CCC. The comparison of plants by the average number of internodes gave interesting results: the plant height was made up by 33.9 internodes in the control plants, by 50.6 in the choline treated and 41.7 internodes in the CCC treated plants. This suggests that the increase in the plant height as a response to choline treatments was not caused by the elongation of the internodes, since each treatment resulted in shorter, but a much higher number of internodes (Table 1). The contrasting effects of CCC are clearly seen in Fig. 1 too, which besides the control (1) shows the results of treatments at concentrations of 1000 (2), 2000 (3) and 4000 (4) ppm. We note here that the lateral shoots of the control and choline treated plants as well as of seedlings treated with 500 ppm CCC showed a normal development and growth, while in the seedlings of treatments (2), (3) and (4) CCC had an inhibitory effect on the lateral shoots too.

Table 2

Effects of choline analogues on the total carbohydrate and crude protein content in shoots and roots of wild pear seedlings (in dry matter percentage)

Treatment ppm	Total carbo-hydrate %		Crude protein %	
	shoot	root	shoot	root
Control	30.60	30.00	9.58	13.31
Choline				
500	30.00	29.85	8.93	14.60
1000	29.25**	28.20*	8.46	12.32
2000	29.55*	28.95	9.11	13.77
4000	30.45	29.85	8.65	14.70
CCC				
500	30.60	29.25	10.04	14.24
1000	30.60	28.65*	9.58	16.10**
2000	30.75	33.60***	11.91**	17.20***
4000	30.60	33.15***	13.77**	20.30***
L.S.D. 1%	1.23	1.97	2.80	2.24

* p = 5%
*4 = 5%
** p = 1%
*** p = 0.1%

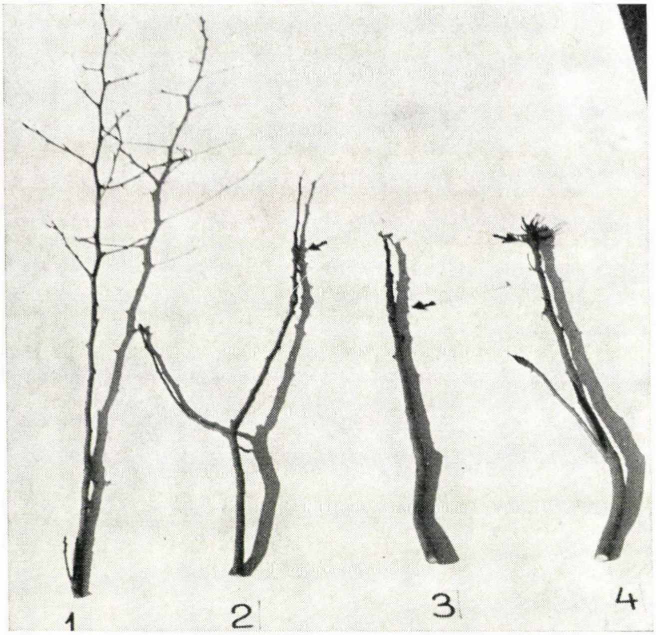

Fig. 1. Rosette form caused by CCC in wild pear (1 = control, 2 = 1000 ppm, 3 = 2000 ppm and 4 = 1000 ppm (1970)

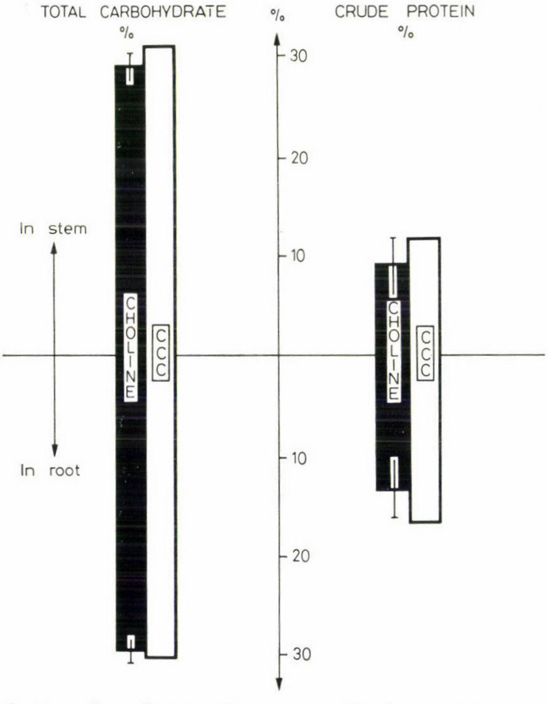

Fig. 2. Total carbohydrate and crude protein contents (in dry matter percentage) in wild pear seedlings (1970)

The results obtained in the analysis of shoot- and root components are summed up in Table 2. It was conspicuous that the choline treatments only had an inhibitory — if any — effect, especially on the total carbohydrate content. CCC displayed a much higher effectivity, significantly increasing the total carbohydrate content in the root when applied at concentrations of 2000 and 4000 ppm. The crude protein content showed a very interesting trend: CCC unanimously resulted in stimulation. CCC at 4000 ppm concentration increased the crude protein accumulation in the shoot almost by 50 per cent. Roots of untreated and choline treated seedlings showed hardly any difference; CCC again had an intensive effect which was quite new in the seedling studies (Table 2).

The comparison between the effects of choline analogues on the value of the components will be more clear if we give a summarization of the results (Fig. 2). By the end of the vegetation both chemicals increased the amount of total carbohydrate and crude protein in the root; a tendencially higher effect of CCC compared to choline was demonstrated in each case, while it had a significantly higher effect on the total carbo-hydrate content of the shoots and crude protein content of the roots, than choline.

Discussion

Our present study has confirmed the result obtained for one year old pear seedlings, that under the influence of CCC the carbohydrate accumulation somewhat increases (Surányi 1970b). However, a much higher effect was shown in the crude protein content as a response to all concentrations. In the absence of experimental results we can only give hypothetical explanations. Bokarev—Ivanova (1971) published the results of thorough investigations on choline and betain analogues. Of them we only mention here the influence of chlorocholine and CCC on the free amino acid content. As a result of CCC treatments the leaves of *Solanum tuberosum* contained 27 per cent, and those of *Solanum schreiteri* 138 per cent more free amino acid than the leaves of plants treated with chlorocholine.

Paxton—Mayr (1962) made an interesting statement, namely, that an increased protein accumulation may take place from choline compounds. Accordingly, from choline serin and glycocoll can be produced, which oxidize in betain. Betain is methylized by homocistein, and methionin is produced which is then transformed into glycocoll. So, if we accept this theoretical pathway, we can regard the different effectivity of CCC and choline as the different reactivity of a stable and a less stable compound, that is, CCC can more easily enter the Paxton—Mayr model.

Since at present we have no CCC treated seedlings of different age available for deciding the after effects of treatments on juvenility, we cannot give any definite answer in connection with the present experiment either. However, the high crude protein content suggests that CCC can be less efficiently used than expected in turning the seedlings into bearing earlier. The considerable amount of protein in the shoot and root seems to indicate the "preparation" of plants for the next year, that is, the accumulation of assimilates did not show the expected trend, although, as a whole, an increased intensity of photosynthesis can be concluded on. All this is confirmed by the fact that in the year following the treatment, i.e. when the seedlings are three years old, they completely overcome the growth inhibition caused by the CCC, and the crude protein content is in correlation with the next year's growth ($r = +0.631$ and $p = 1$ per cent).

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 51—54 (1976)

EFFECT OF DIFFERENT LEVELS OF DIETARY PROTEIN ON DIGESTIBLE ENERGY IN LAMBS

By

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Digestible energy was determined in a digestion study with lambs, during growth, fed on different levels of dietary protein. The percentage of digestible energy was decreased by increasing the level of dietary protein.

Introduction

The need for sufficient readily available carbohydrates for nitrogen utilization has been demonstrated and well understood. However, Putnam *et al.* (1966), found that the apparent digestibility of organic matter, energy and nitrogen free extracts was the highest at the medium level of energy intake, and the digestibility of crude protein, crude fiber and ether extract was the lowest when animals were fed at high energy levels.

The results of Schelling *et al.* (1967) showed no significant differences in the average daily gain of lambs fed with different dietary protein levels. Moreover, Mahmoud (1972), found that the increase of the protein level to 19.5 per cent in lambs' rations resulted in significant decrease in the growth rate. Thus the utilization of dietary nitrogen was decreased by increasing the level of dietary protein.

Material and Methods

Twelve Hungarian-Merino wethers were used in this experiment. The animals were about three months old and weighed 20 kg. They were divided into four groups at random, the groups contained three animals each. Four levels of crude protein (13.8; 16.5; 19.5 and 23.5 per cent) were fed in pelleted mixed rations to the first, second, third and fourth group, respectively. The diets provided equal daily energies. The gross energy (Kcal per g concentrate) ranged from 4.8 to 5.7. Concentrates were fed together with wheat straw twice a day in a level near to ad libitum. After 86 days feeding, the lambs were of 32 kg live-weight. In the N-balance trials faeces was collected during eight successive day safter one week adjustment period. Feces of 4—4 days collection was used separately for the determination of digestibility.

Gross energy was determined using a Berthelot—Mahler type bomb calorimeter with 20 atm O_2 pressure.

Results

Gross energy, energy output in feces and digestible energy are shown in Table 1. The gross energy intake was about the same for each group. However, the energy output in feces was higher in the third and fourth group than

Table 1

Daily gross energy intake, energy output in feces and digestible energy for the different groups

Group	No. of animal	Collection period	Gross energy Kcal	Energy in feces Kcal	Digestible energy Kcal
I	19	A	4815	1105	3710
		B	—	—	—
	25	A	5241	1346	3895
		B	5335	1264	4071
	28	A	5226	1550	3676
		B	5335	1422	3913
II	29	A	5623	1696	3927
		B	5594	1798	3796
	38	A	5613	1760	3853
		B	5584	1836	3748
	115	A	5451	1673	3778
		B	5484	1694	3790
III	51	A	5562	1688	3874
		B	5791	1907	3884
	58	A	5621	1965	3656
		B	5725	1982	3743
	59	A	5637	1811	3826
		B	5611	1951	3660
IV	69	A	—	—	—
		B	5593	1858	3735
	85	A	5379	1818	3561
		B	5632	1979	3653
	91	A	5389	1947	3442
		B	5574	1769	3805

A) Mean of the first 4 days collection period
B) Mean of the second 4 days collection period

in the first and the second one. The mean apparent digestibility of gross energy was calculated and is given in Table 2.

There was a tendency for the apparent digestibility of gross energy to decrease with increasing dietary protein concentration. The digestibility of gross energy decreased from 74.7 to 66.1 per cent with increasing the dietary protein from 13.8 to 23.5 per cent. However, Andrews—Ørskov (1970) found that for lambs kept on high feeding levels, the apparent digestibility of gross energy increased as protein concentration increased. The differences between our experiments and that of Andrews and Ørskov may be partially explained by the older age of our experimental lambs (32 kg versus 19 kg).

As shown by Mahmoud (1972), increasing the protein level up to 16.5 per cent of the lambs' ration resulted in a decrease of the growth rate. The lower growth rate may be due to the lower efficiency in energy utilization. The nitrogen was available in excess of requirement. The excess of nitrogen was converted mainly to urea and the blood urea nitrogen was high. Juhász—Szegedi (1972) suggested that additional energy was required for the urea resynthesis in the liver. Also the results of Stobo *et al.* (1967) indicated clearly the importance of an adequate supply of energy in the diet of the ruminant calf if maximum live-weight gain is to be achieved. In planning this experiment, we aimed to fulfil two demands. First, to provide equal dietary energies in each group. Secondly, to use a high level of energy compared to the different dietary protein levels.

Table 2

Mean apparent digestibility of gross energy (DE%) as affected by the level of dietary protein

First group[1]		Second group[2]		Third group[3]		Fourth group[4]	
No. of animal	DE%	No. of animal	DE%	No. of animal	DE%	No. of animal	DE%
19	77.0	29	68.8	51	68.2	69	66.8
25	75.3	38	67.9	58	65.2	85	65.5
28	71.8	115	69.2	59	66.5	91	66.1
Average of three animals							
	74.7		68.6		66.6		66.1

1. 13.8% dietary protein
2. 16.5% dietary protein
3. 19.5% dietary protein
4. 23.5% dietary protein

Mean of 8 days collection period
Mean of only 4 days collection period

Andrews—Ørskov (1970) found that the optimum dietary protein concentration for growing lambs between 16 and 40 kg body weight was about 17.0; 15.0 and 11.0 per cent when the mean digestible energy intake was 3.0; 2.6 and 2.1 Mcal/day, respectively. The mean digestible energy intake in this experiment was 3.9; 3.8; 3.8 and 3.6 Mcal/day for the first, second, third and fourth group, respectively.

It is suggested that the lower efficiency in energy utilization found in the third and fourth group may be due to the effect of the high level of the dietary protein and not to energy deficiency. However, the percentage of digestible energy was a good indicator in evaluating the different rations.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 55—63 (1976)

TRANSLOCATION AND INCORPORATION OF ^{14}C-LABELLED MORPHACTIN IN APPLE SPURS

By

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^{14}C-labelled chloroflurenol (active agent of EMD-IT 3456) was applied on the leaf of detached spurs of apple trees (variety: Jonathan). Under similar conditions, active solution was applied into the pedicel cavity of fruits to be found on the spurs. Practically uniform distribution was found in all tissues of the leaf, with a slightly lower activity in the spongy parenchyma. In petiole cross-sections frequently the phloem displayed the strongest labelling. Incorporation into bud tissues appeared to be relatively uniform. In the seedlet both the endosperm and the integument display significant incorporation. The simultaneously performed macroautoradiography shows that within five hours' post treatment there was no translocation into mature leaves opposite to the treated leaf of the spur. The applied morphactin is easily incorporated and freely translocated both in a basal and an acropetal direction.

Introduction

The numerous publications dealing with the components of the biological activity of morphactins are summarized in comprehensive reviews (ZIEGLER 1970, SCHNEIDER 1972a, 1972b). Consequently, it cannot be the aim of this report to make a review on the action mechanism and practical utilization of morphactins. Reference to some fundamental reports, however, is necessary.

It is about 10 years that the first report has summarized this new group of synthetic growth regulating substances known under the collective name morphactin (SCHNEIDER 1964). Morphactins have proved to be highly effective growth retardants, moreover, RINGE—DENFFER (1967) give an account of their general development inhibiting properties. They have the particular effect of changing the geotropic and phototropic response of plant organs (KHAN 1967, KRELLE—LIBBERT 1968a) and inhibit endogenous auxin transport (KRELLE—LIBBERT 1968b, TOGNONI—ALPI 1969, PILET 1970). It seems due to this latter effect that the habit of morphactin-treated plants is changing through the moderation of apical dominance. Attention was soon turned to the relation with endogenous growth hormones.

The earlier presumption that morphactins are competitive gibberellin antagonists (ZIEGLER *et al.* 1966) has not been ascertained by later experiences. Disturbance of the IAA biosynthesis (VOGT 1968) is one of the explanations of the decrease in IAA-level after application of morphactins but the latter are no real IAA-antagonists (Ziegler 1970). At the same time, affinity of the IAA-oxidase directed on the enzyme substrate and reaction speed are likewise increasing (Treichel 1971). According to the author's own data (Bubán, 1974), IAA-oxidase activity increases in morphactin treated apple tree leaves but the increase in activity depends on the age of the leaves. According to Pianiazek *et al.* (1970) morphactin effects observed in apple seedlings are a consequence of the interaction of endogenous auxin and cytokinins.

The sometimes drastic regulation of the formation and development of different plant organs offers a possibility to the practical utilization of morphactins. Examples of this are to be found in fruit, vine and ornamental plant growing as well as in the field of plant pathology. Partly contrary with a first report (Bubán *et al.* 1969) it was found that certain morphactins stimulate flower bud formation in apple and peach trees (Bubán 1972a, Bubán *et al.* 1972) and — in accordance with several years' experiences of Hugard *et al.* (1972) — can be used for the chemical fruit thinning of peach trees (Bubán 1972b). In the present work the movement of a morphactin in plant organs was examined with the aim of rendering further experiments more detailed.

Material and Method

The morphactin used in the experiments was ^{14}C-labelled chloroflurenol (active agent: methyl-2-chloro-9-hydroxy-fluorene(9)-carboxylate) with spec. activity of 38.1 μCi/mg. The substance solved in ethanol was applied on leaves and fruits of detached spurs of Jonathan apple trees on M 4 root stock (date: 27th of June). On each spur two developed leaves were left, one of them was treated. At the time of the first experiment (5th of May) the younger leaflets had not been removed. The labelled active agent was applied on the basal third of one of the mature leaves, in a lanolin ring of about 1 cm in diameter in 100 microlitre solution. In the treatment of the fruits on the spurs the lanolin ring was made around the fruit pedicel, on the edge of the pedicel cavity. On each spur either the leaf or the fruit was treated, and the activity applied on each spot was 6 μCi. The actual test material was the upper third and the petiole of the treated leaves, respectively and the seedlet of treated fruits which were collected 3 to 5 hours post application.

The collected samples were quickly deep-frozen and stored at —20 °C until processing. For microautoradiography deep-frozen sections were prepared in a cryostat (System Dittes-Duspiva). Sections were prepared at —18 °C, average section thickness was 20—30 micron, depending on the plant organ. The sections were left overnight in the cryostat, so that the ice sublimated. Kodak AR-10 stripping film was then fixed on the section without previous stretching on water. Exposure time was 10—12 weeks, development (D-19) and fixation (Unifix) lasted about 5—10 minutes. In addition macroautoradiographs were prepared using X-ray film.

Results

Distribution of activity in tissues of the treated leaf. The distribution in the leaf cross section of ^{14}C-labelled morphactin applied on the basal third of the leaf blade can be characterized as follows: On the autoradiographs it is clearly visible that there is no zone in the leaf cross section either below or above the point of application which would have caused a particularly strong blackening of the film (Fig. 1). During the relatively short experimental time of 3 to 5 hours no essential metabolization of morphactins could have taken place. With the presumption of this the autoradiographic observations can be interpreted in a way that the distribution of the morphactin applied is fairly uniform in the leaf cross section. There is no tissue inside the leaf which would show a particularly strong accumulation of the morphactin, with the exception of the veins. Frequently, blackening is slightly weaker above the spongy parenchyma than above the pallisade cells. Probably, this phenomenon can be explained by the fact that a relatively low number of cells are to be found in the spongy parenchyma. Due to the relatively large section thickness and the fact that there was no ^{3}H-labelled morphactin available the intracellular localization could not be achieved in the way SCHÜTTE *et al.* (1972) could realize it. In their experiments it seemed that morphactin is localized mainly in the cytoplasm of cell.

Autoradiographic examination of the pedicel cross section of ^{14}C-labelled morphactin treated leaves after three and five hours experimental time, respectively, indicated that the translocation from the leaf blade had started. In addition, a significant lateral transport could be observed in the petiole. Consequently, radioactivity was not restricted only to the transporting elements, and the labelling had already translocated into the neighbouring tissues too. A stronger blackening could frequently be observed in the phloem area than in the neighbouring tissues. Unequivocal identification of the transporting elements out of leaves was not achieved, due to the length of the given experimental time and the strong lateral transport, respectively.

Incorporation into the terminal bud after leaf treatment. The movement of morphactin applied on the leaf into the terminal bud could be established. The distribution of activity inside the bud was relatively uniform. The bud base, leaf primordia and apex caused nearly equal blackening of the film indicating that there was no tissue accumulating the morphactin in a preferential way. It seems evident, however, that morphactins act specifically in certain tissues inside the apex as can be concluded from the fact that flower bud formation can be increased by morphactins (BUBÁN 1972a).

Accumulation in the seed of morphactin applied on the fruit surface. The morphactin applied on the fruit surface can be demonstrated by autoradiography a few hours afterwards in the seeds. Accumulation occurs mainly in the integument and endosperm (Figs 2 and 3), and to a small extent in the nucellus. No suitable sections could be achieved from embryos for the autoradiographic demonstration of incorporation into the embryos, which were still small at that stage of seed development. Since, however, the strong accumulation of certain substances, e.g. ^{32}P, ^{45}Ca, ^{14}C-labelled sugar in the embryo is known (e.g. STÖSSER 1970, STÖSSER—NEUBELLER 1970) it can be assumed

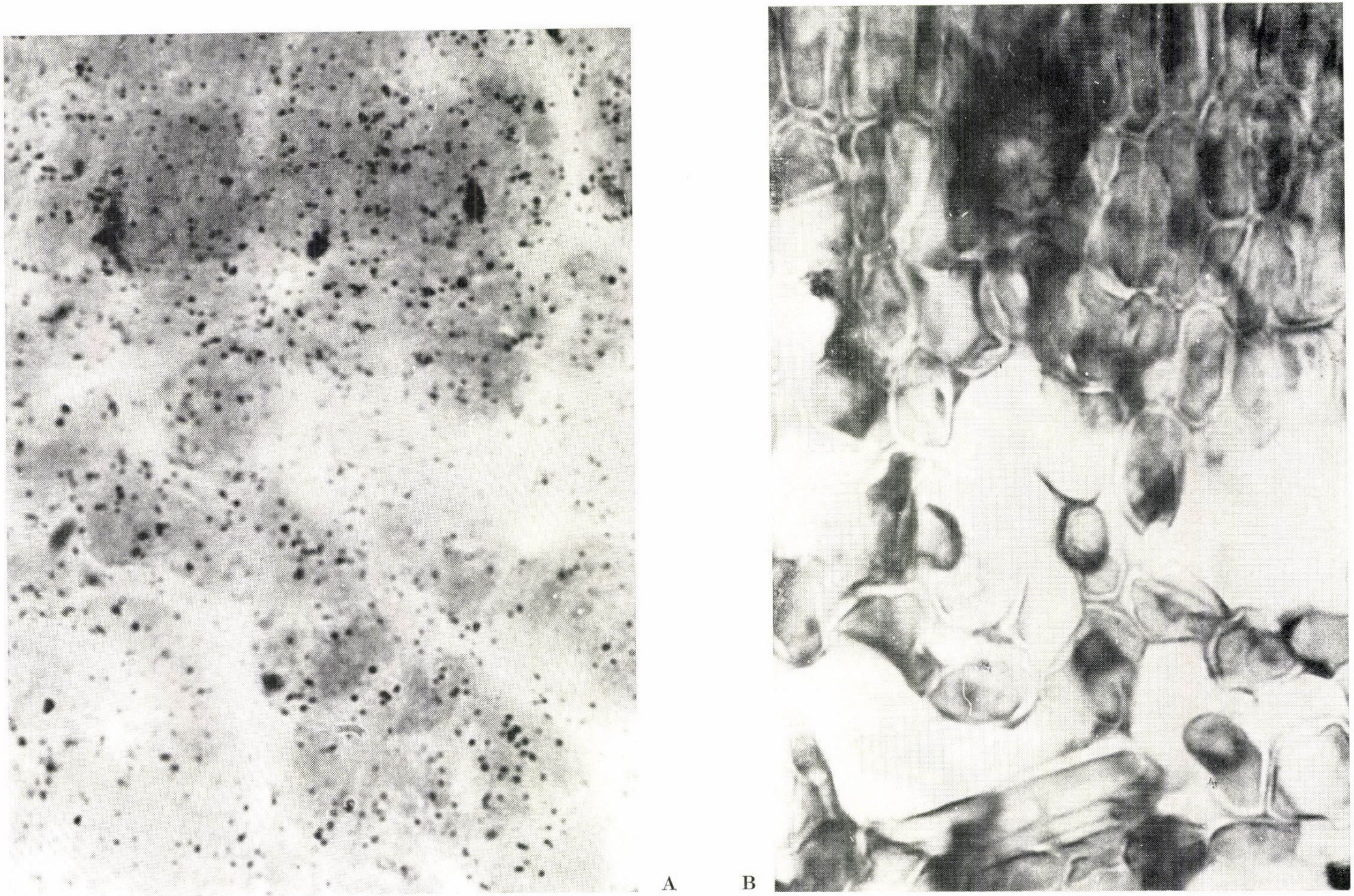

Fig. 1. Distribution of ^{14}C-labelled chlorflurenol in a leaf above the point of application three hours post application (6 μCi were applied on the lower third of the leaf blade). A) Autoradiography, B) histological deep-frozen section corresponding to A)

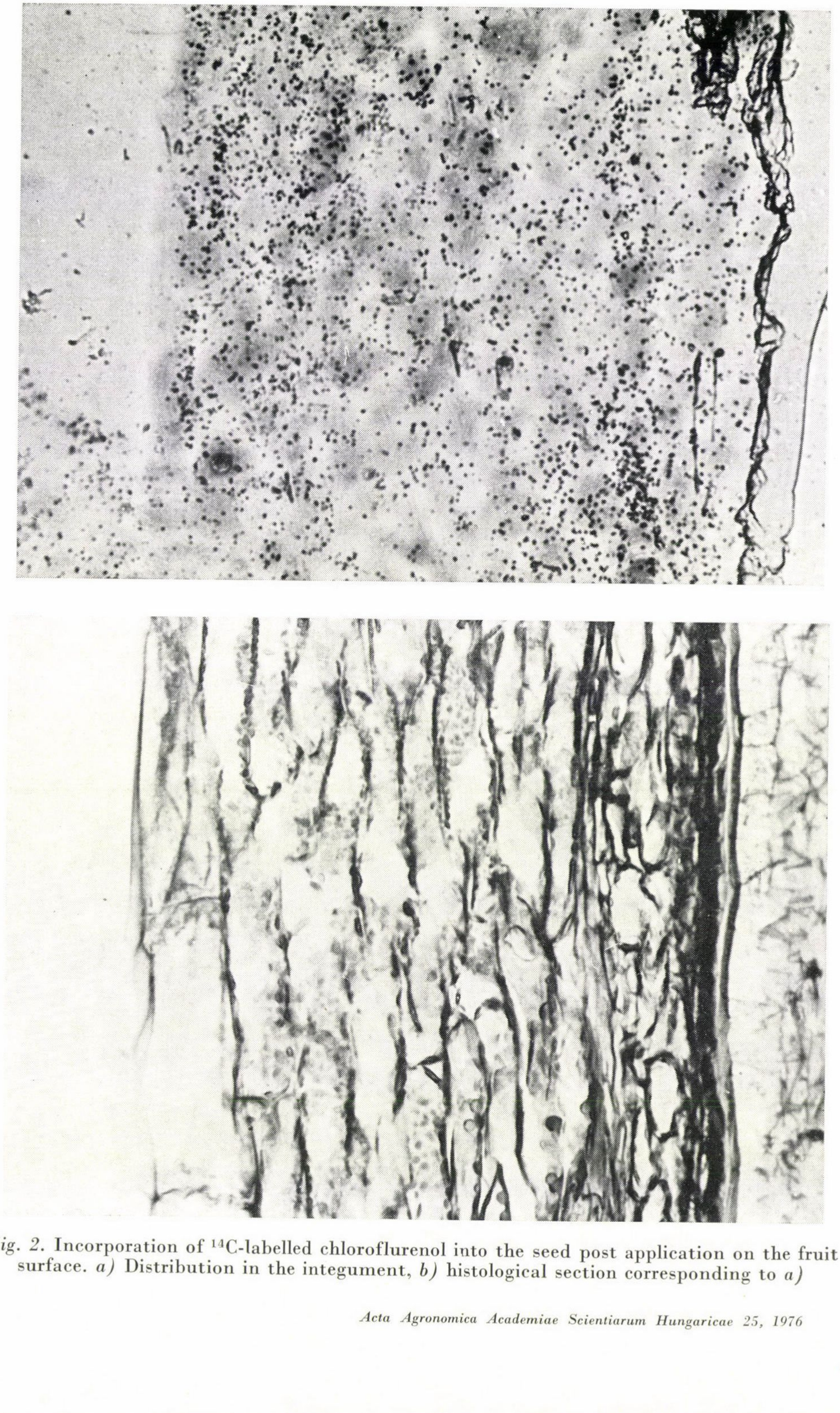

Fig. 2. Incorporation of ^{14}C-labelled chloroflurenol into the seed post application on the fruit surface. *a)* Distribution in the integument, *b)* histological section corresponding to *a)*

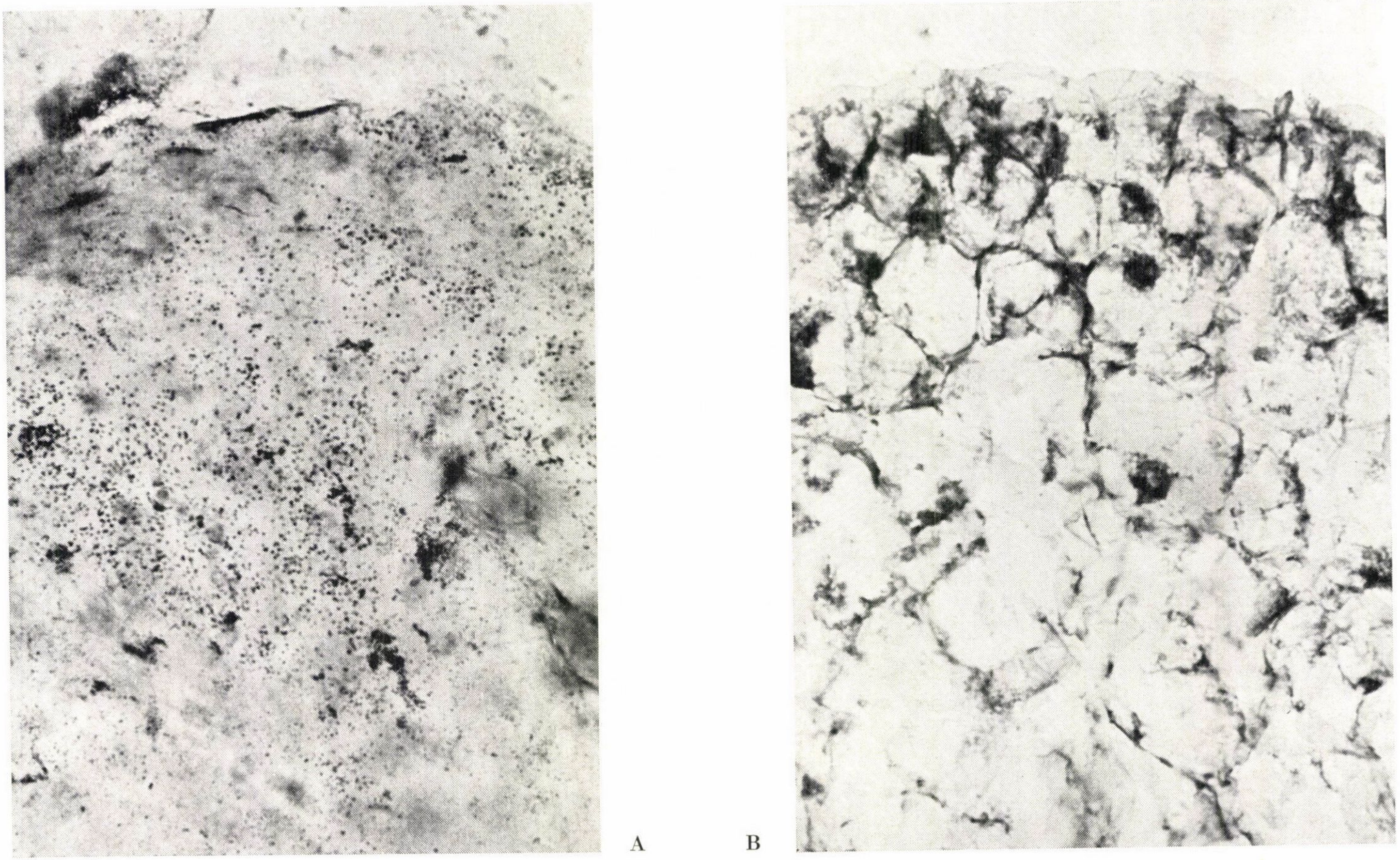

Fig. 3. Incorporation of ^{14}C-labelled chloroflurenol into the seed post application on the fruit surface. A) Localization in the endosperm, B) histological section corresponding to A)

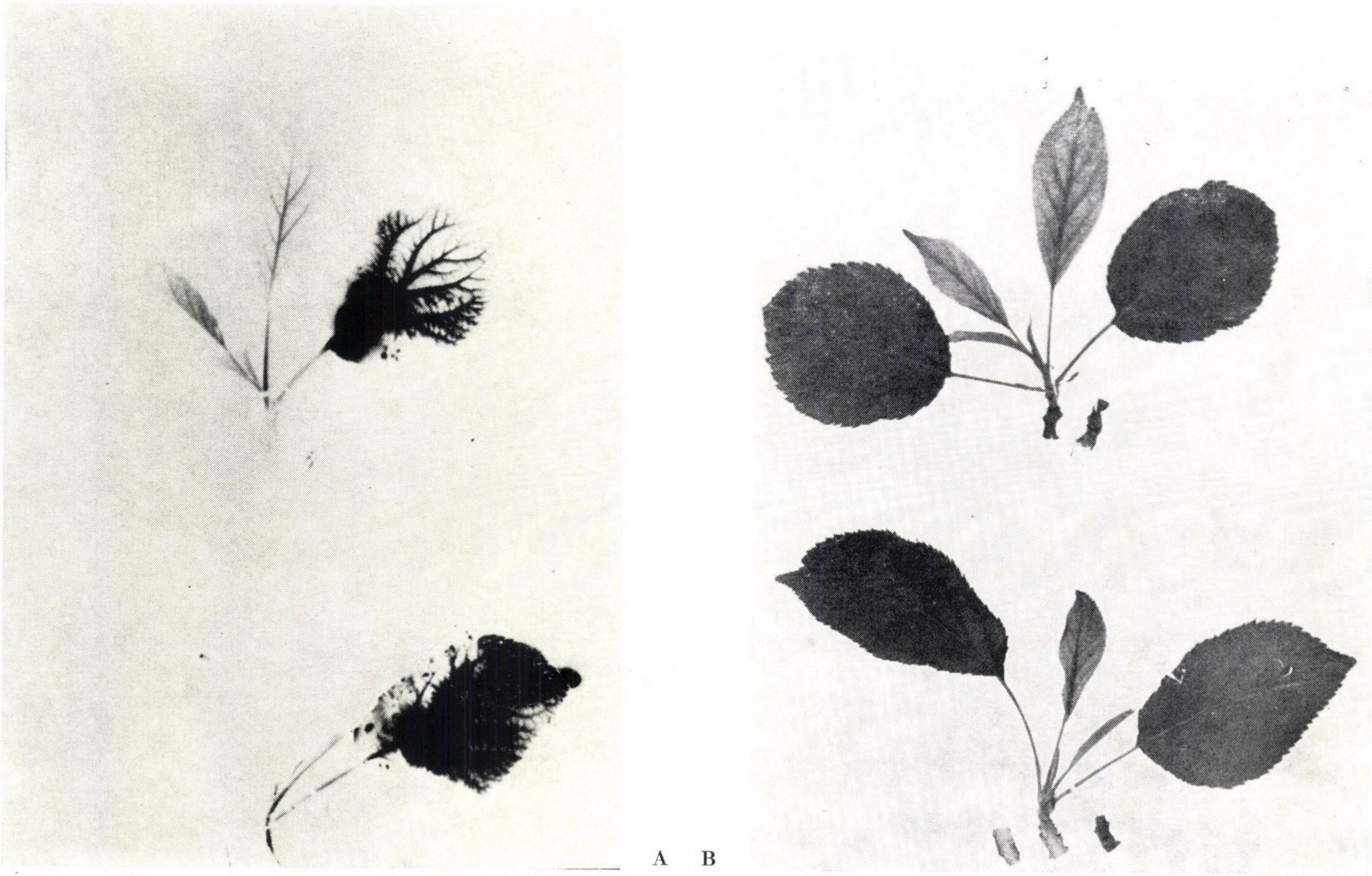

Fig. 4. Distribution of ^{14}C-labelled chloroflurenol in treated and neighbouring untreated young leaves of a spur. A) Autoradiography, B) specimen

that by incorporation into the endosperm an accumulation takes place in the embryo too.

Macroautoradiographic observations. After application of the active agent within a short time the whole surface of the treated leaf shows activity. The movement occurs in the veins of the leaf. In the developed leaf opposite to the leaf where the label had been applied no morphactin could be demonstrated five hours' post treatment. A high concentration could be found, however, in young leaflets of terminal position on growing spurs examined in the experiment with young spurs (Fig. 4).

Discussion

The used morphactin is easily incorporated and freely translocated both in an acropetal and a basipetal direction. It shows a relatively uniform distribution in the leaves and buds of the apple tree. Within a given organ no stronger accumulation can be established in a certain tissue. From the fruit surface it moves to the seeds.

As mentioned in the introduction, after morphactin treatment of apple and peach trees a flower bud stimulation was observed. From this point of view it is remarkable that labelling soon appears in the young and growing leaflets of spurs examined in an early stage of development. In seedlets of treated young fruits the accumulation was likewise. These organs, however — producing centres of endogenous hormones — have an inhibitory effect on flower formation in apple and peach trees. It seems probable that the flower bud formation increasing action of morphactins is based on the stimulation of IAA inactivation and polar transport inhibition, respectively. The possibility of the compensation of various gibberellin effects cannot be excluded either. Further examinations are needed, for example, the subcellular localization of morphactins, and the effect on endogenous phytohormones.

Acknowledgements

The authors wish to thank Dr. G. Mohr (Celamerck, Ingelheim, Germany-West), for the supply of morphactin. One of the authors, Dr. T. Bubán, is expressing his thanks to Prof. Dr. Ottilie Zeller (Department of Botany, Universität of Hohenheim) for her interest and assistance related to the experiments.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 65—67 (1976)

EFFECT OF DIFFERENT SPORE NUMBER CLAVICEPS PURPUREA CONTAGIA ON THE YIELD OF ERGOT

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Experiments were performed to find out whether by increasing the spore number of the generally used *Claviceps purpurea* suspension containing 5×10^3 spores per mm^3 a higher yield of ergot can be attained. According to the experiments carried out at Rákoskeresztúr the yield increased significantly, in the case of applying a contagium containing $40 \times 10^3/\text{mm}^3$ spores in the first, and $10 \times 10/{}^3\text{mm}^3$ spores in the second experiment.

Introduction

Since the amount of ergot produced naturally is far from satisfying the demand of the pharmaceutical industry, some authors have tried to attain a higher yield by artificial infection.

Conidiospores of *Claviceps purpurea* grown on culture media were used by Bonns (1922), Falck (1922), Kirchhoff (1929), Krebs (1936), Békésy—Garay (1960).

According to the investigations of Békésy (1955, 1956) and Kybal—Vavrusková-Zadinová (1955) a suspension suitable for infection has to contain $5 \times 10^3/\text{mm}^3$ spores. However, the experts of the "Herbaria" National Co-operation of Medicinal Plant and Cocoon Trade observed in sufficient ergot yields in artificially infected stands in several cases. We wished, therefore, to examine the question whether by increasing the number of spores a more favourable yield can be attained.

Material and Method

The experiment was performed with the ergotamin strain isolated by Békésy at the Research Institute of Medicinal Plants and used for large-scale ergot production in that year.

The cultures were produced by inoculating a slanted agar-agar medium with sterile pieces of the inner pseudoparenchyma tissue of the sclerotium. The test tube cultures were propagated on malted agar-agar medium in *T.* kolle glasses. Suspensions containing 5×10^3, 10×10^3, 20×10^3, 40×10^3 and $80 \times 10^3/\text{mm}$; spores, respectively, were prepared with a sterile physiological salt solution. The cultures were 3—12 weeks old, the germinative ability of the spores was 70 per cent (Soós 1969).

The field experiments were laid out at Rákoskeresztúr, in the middle of an about 20 ha rye field of the "Összefogás" Co-operative Farm, in plots of $6 \times 6 = 36\ \text{m}^2$ each, according to Sarkadi (1962) in a Latin square design with 4 replications.

On the advice of BÉKÉSY (1956) infection was carried out when the tip of the rye ear emerged from the leaf sheath. The first experiment was performed between 12th and 14th May 1964, the second on 10th May 1965. Infection was carried out by hand with cards described by BÉKÉSY (1947).

Harvesting of the ergot produced on the field plots was made by hand with a collecting apron described by ANDRUSKO (1956).

Results

The kg/ha quantities of ergot produced in experiments performed with *Claviceps purpurea* contagia of different spore number are presented in Table 1.

Table 1

Ergot yields of rye infection experiments performed with Claviceps purpurea contagia containing different numbers of spores

Site and number of experiments		Treatments	Number of spores used for infecting rye, mm^3	Yield	
				kg/ha	%
Rákoskeresztúr	I	Spores washed off an agar surface	5.000	26.6	100.0
			10.000	50.0	187.7
			20.000	79.3	297.9
			40.000	89.7	337.2
			80.000	81.5	306.3
			s.d.$_5$		73.6
	II	Spores washed off an agar surface	5.000	94.2	100.0
			10.000	125.8	133.6
			20.000	127.0	134.9
			40.000	133.8	142.1
			s.d.$_5$	15.3	16.2

The yields were evaluated with variance analysis. The values are given in percentage too. When comparing the yield amounts the yield attained with a contagium containing $5 \times 10^3/\text{mm}^3$ spores was taken for 100 per cent.

The evaluation of the data has revealed significant yield differences in both experiments at $P = 0.1\%$. In experiment I the yield significantly increased up to the variant containing $40 \times 10^3/\text{mm}^3$ spores. Further increase in the spore number did not result in higher ergot yields.

In experiment II the increasing tendency of yield with the increase of the spore number could be demonstrated, but significant results were only obtained with a contagium containing $10 \times 10^3/\text{mm}^3$ spores.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 69—78 (1976)

MICROELEMENT AND HABITAT REQUIREMENTS OF VINCA MINOR L.

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The authors studied *Vinca minor* plants originating from 57 habitats and their soils in order to get information on their nutrient and habitat requirements. They pointed out that in the natural habitat of the plant the acidic forest soils are dominant whose essential characteristics are: low humus stability value and high mobility iron and manganese content. The plant is characterized by an iron, manganese and copper content exceeding the average of the Hungarian natural flora, combined with a lower than average sodium and molybdenum concentration. The difficulties of field production arise from the inability of the plant to satisfy its own specific nutrient requirements, whereby it is carried away by fungal diseases due to its reduced resistance. On soils of high calcium content ammonium sulphate and ammonium dihydrogenphosphate fertilizers have to be applied to increase the amount of the available manganese, as one of the chemotaxonomic characteristics of *Vinca minor* is to take up considerable quantities of this element.

Introduction

With a view to a more perfect fulfilment of the social requirements many wild plant species have recently been introduced in cultivation. Such a process has never been devoid of problems. Reserpine occurring in the tropical plant species *Rauvolfia* occupies an important place among the plant origin drugs which decrease the blood pressure. *Vinca minor* L., a plant occurring spontaneously in Hungary, similarly belongs to the family *Apocynaceae*.

This evergreen chamaephyton of Mediterranean origin occurs in Hungary mainly in hornbeam groves and oak-forests. By the industrial production of vincamine — the main alkaloid of the plant — SZÁSZ (1963), and by the demonstration of its structure CLAUDER (1963) won distinction for the Hungarian researchers. In connection with the problems of cultivation MÁTHÉ *et al.* (1967), HUBAY (1967), MÁRK *et al.* (1969) and NAGY *et al.* (1970) studied the habitat requirements of the plant, and the relationship between its alkaloid content and disease resistance. The above researches clarified many consequences of taking the plant under cultivation as well. So it has been found that this shade loving plant when transferred to the open field redoubles its alkaloid concentration observed in its original habitat. Beside this economically favourable change severe leaf spots as well as shoot and stem decay occurred in the planted stands. The disease destroyed more than half of the field cultures, and the weedplants overgrowing the less closed stands rendered even the reduced drug yield valueless. Since many pathogenic fungi had been isolated (e.g. *Phoma vincae minoris*) from the diseased plants combined fungicide treatments were carried out with a great diversity of chemicals. As all combinations proved ineffective, NAGY *et al.* (1970) declared that "the infection is not primarily of fungal origin; the shoot- and stem decay is caused by the joint effect of unfavourable ecological factors and pathogenic fungi". MÁTHÉ (1968) arrived at the same conclusion: "in my opinion the increased alkaloid and/or active agent content was caused by the unusual, less favourable conditions". In spite of all these statements, of the ecological factors the agrochemical properties of the habitat and the nutrient uptake of the plants have remained almost totally unknown with the exception of MÁTHÉ's (1965) observation that the amount of vincamine produced is the most favourable when the pH value of the soil is between 6.0 and 7.5. After such preliminaries we thought it worth specifying the nutrient and habitat requirements of *Vinca minor* by measuring a number of characteristic features.

Material and Method

Our results are based on the plant and soil samples collected by Kaposi between 11 and 26 October 1971 mostly from spontaneous habitats. The plant samples consisted of 15—20 healthy, fully developed shoots each, from which the dust and soil particles had been removed according to the requirements of the microanalysis (Tölgyesi 1969). The soil samples were taken from the total root zone immediately below the collected plants. This does not cause any difficulty as—according to Máthé—99 per cent of the root-system is found in the upper 10 cm layer. After a perchloro acid destruction the air-dry plant samples were determined partly with colorimetric (P, Fe, Cu), partly with atom absorption (K, Ca, Mg, Na, Mn, Zn) methods. After the material had been reduced to ashes the boron and molybdenum contents were subjected to colorimetric examinations. The colorimetric measuring was carried out with a Zeiss Spekol colorimeter. The phosphorus was measured in the form of molybdate, while the iron was determined with dipyridyl, the copper with diethyl-dithiocarbamate, the boron with carmine and the molybdenum with rodanide. The instrument used for atom absorption was: Perkin-Elmer 290/B. Apart from some data of basic soil analysis (1) the available nutrients were determined by extraction with 0.1 normal HCl at room temperature for 12 hours, while the total acid soluble nutrients by extraction with hot perchloro-acid. In the statistical evaluation we followed Sváb's (1967) directives. For the sake of lucidity the nutrient and habitat requirements are only outlined for *Vinca minor*. General soil — plant correlations established in the course of processing the data will be discussed later with other models.

Results

Characteristic features of the soils of habitats. The examined parameters of the natural soils of *Vinca minor* (Table 1) are characteristic of the forest soils: they have low pH values, low sodium and molybdenum contents, and with the exception of the South-Transdanubian samples low calcium contents too. Their iron and manganese contents, on the other hand, are more readily available than in the meadow soils. The humus stability value (Q) of the typical habitats is always below 1 (HARGITAI 1955).

Table 1

Basic analysis data of Vinca minor soils. Comparison between their nutrients soluble in hot perchloro-acid ("total") and those soluble in 0.1 n HCl at room temperature ("soluble") on the one hand, and the nutrients of cultivated soils in two farms, on the other

Series	Number of sample	pH		Q		K	Ca	P	Mg	Fe	Mn	Zn	Cu	Mo
		H_2O	KCl	450 μu	650 μu	g/kg				mg/kg				
1	57	6.28	5.53	1.31	1.23	2.96	9.6	0.57	3.9	18.100	720	49	9.1	0.38
2	23	5.48	4.96	1.35	2.12	5.90	5.9	0.81	4.6	26.100	610	43	26.5	0.35
3	30	7.90	7.40	3.17	3.22	3.47	54.4	0.69	12.8	14.700	374	34	18.1	0.13
4	57					0.18	7.1	0.13	0.96	203	335	9.5	2.7	
5	23					0.18	3.5	0.38	0.97	230	190	3.3	5.4	
6	46					0.12	17.2	0.21	2.28	8	68	3.2	0.2	

Series

1 Basic analysis data of *Vinca minor* soils, and nutrients soluble in hot perchloro acid
2 Basic analysis data of soils under field cultivaton at the foot of the Mountains Mátra (Pásztó), and nutrients soluble in hot perchloro acid
3 Basic analysis data of soils under field cultivation in the Danube—Tisza Mid-region (Vány), and nutrients soluble in hot perchloro acid
4 Nutrients of *Vinca minor* soils soluble in 0.1 n HCl at room temperature
5 0.1 n HCl-soluble nutrients of soils under field cultivation at the foot of the Mountains Mátra (Pásztó)
6 0.1 n HCl-soluble nutrients of soils under field cultivation in the Danube—Tisza mid-region (Vány)

The evaluation will be easier if we compare the soils of *Vinca minor* with an acidic brown forest soil originating from 23—23 plots under field cultivation at the foot of the Mountains Mátra (Pásztó) and a shallow sodic humous sandy soil from the Danube—Tisza Mid-region (Felsővány). It can be established that the total potassium content of the *Vinca* soils is lower than that of the cultivated soils, while its easily available part is largely the same. The average calcium content is raised above the level of an average forest soil only by the soils of the South-Transdanubian habitats. A large part of this amount is, however, present in an available form. Both the phosphorus content and its availability are lower than in the cultivated soils. On the *Vinca* soils more or less exposed to eluviation little sodium is absorbed. The result is in agreement with the data of the plant analyses. The iron content runs parallel with the stickiness of the soil. The iron contents of the examined samples correspond to the level of medium sticky soils, its availability is high. As to the total — and particularly the available manganese content, the examined soil samples differ from the meadow soils, and show the characteristic values of the acidic forest soils. The zinc contents are medium, but their availability is high. The *Vinca* soils have a low, medium soluble copper content. This is all the more interesting because the plant takes up a remarkably large quantity of copper even from these small reserves.

Table 2

Composition in the above-ground parts of Vinca minor in an air-dry state

Number of samples	Origin of samples	K	Ca	P	Mg	Na
		g/kg				
57	Average	16.1	7.9	2.56	5.8	0.060
19	From extensive habitat	15.2	7.6	2.35	5.9	0.050
14	From South-Transdanubia	13.4	7.1	2.65	5.3	0.066
25	From West- and North-Transdanubia	16.2	8.0	2.52	6.2	0.064
18	From the Northern Medium-Height Mountains	16.8	8.3	2.54	5.5	0.048

		Fe	Mn	Zn	B	Cu	Mo	Fe/Mn
		mg/kg						
57	Average	314	177	38	38	11.5	0.22	1.77
19	From extensive habitat	273	245	37	39	11.2	0.19	1.12
14	From South-Transdanubia	282	79	30	38	10.6	0.32	3.57
25	From West- and North-Transdanubia	281	240	39	35	11.2	0.19	1.17
18	From the Northern Medium-Height Mountains	387	160	72	42	12.8	0.19	2.41

The nutrient concentration of the plant. In the 57 *Vinca minor* samples collected in the middle of October the average concentration of five macro- and six micronutrients was of the same order of magnitude as the composition of elements in the Hungarian flora (Table 2). The concentrations of potassium, calcium, phosphorus, magnesium, zinc and boron are moderate, while those of iron, manganese and copper higher than the average compared to 1400 wild (and most cultivated) plant species. Sodium and molybdenum are contained in Vinca minor at relatively low concentrations.

The high copper content is a chemotaxonomic characteristic of *Vinca minor* frequently observed in the *Malvales-Solanales* branch (e.g. in plants belonging to the orders *Boraginales, Solanales*); but it is sharply distinguished from them by its high manganese content found in the families of the order *Gentianales*, and seldom occurring in herbaceous terrestrial plants.

Although the nutrient concentration found in the plant cannot always be brought into connection with its nutrient requirement, on the basis of our habitat studies we still think that the inorganic composition of *Vinca minor* is not only a feature characteristic of the species, but reflects ecological demands too. This explains that *Vinca minor* whose spontaneous spreading is restricted to relatively close ecological conditions is a not easily adaptable plant species. As far as we measure its nutrient demand by the extent of its nutrient uptake, it requires first of all an increased availability of manganese and copper. Among the cultivated plant species of Hungary there is not a single one that takes up as much manganese as the *Vinca minor*, and as regards the copper content it competes with the first copper utilizing species (except sunflower). *Vinca minor* is mainly distinguished from the cultivated plants of Hungary by its low molybdenum content, as through a selection for protein and protein-molybdenum ratio most cultivated plants in Hungary contain a considerable amount of molybdenum (TÖLGYESI 1970).

With a view to the nutrient supply it is important to know how much of the individual elements is extracted by the above-ground parts. Although in practice the usual drug yields are only 8—10 q/ha, we still give the results of calculations for a potential 22 q/ha yield (SVÁB 1969). With the exception of nitrogen, the 22 q/ha yield of *Vinca minor* extracts nutrient of a kg order of magnitude only in four elements (potassium: 35.4 kg; calcium: 17.4 kg; magnesium: 12.7 kg; phosphorus: 5.6 kg), while in the case of the other elements less than that (iron: 690 g; manganese: 380 g; sodium: 130 g; zinc: 84 g; boron: 84 g; copper: 25 g; molybdenum: 0.55 g). Insofar as we only have the task of replacing the nutrient content, 73 kg/ha active agent is required to maintain the nutrient equilibrium — except the nitrogen. The peculiarities of the nutrient demand can be revealed without the analysis of the soils if we compare the composition of extensive populations with that of sporadically occurring populations. We used the following method: marked out about one-third of the sites of sample taking where *Vinca minor* covered a larger than 2 cad. yoke continuous area assuming that in these places the plant found its living conditions and the necessary nutrients better than in other places. While there is but a slight difference in 9 elements between the two habitat groups, the iron and manganese contents show opposite tendencies. Consequently, the iron/manganese ratio is lower at habitats favouring the spread of *Vinca minor* (1.12) than on an average of the occurrences (1.77). On the basis of the iron–manganese antagonism *Vinca minor* is thus supposed to be an expressedly manganophilous and — by concomitance — more or less acidophilous medicinal plant.

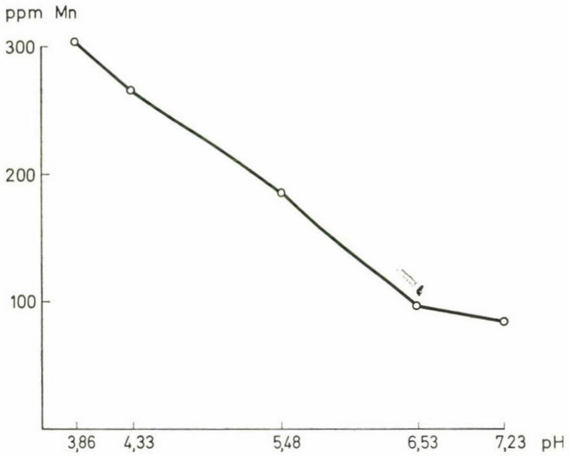

Fig. 1. Relationship between the pH value of the soil and the manganese content of *Vinca minor* L.

The influence of the chemical reaction of soils is shown in Table 3. According to the soil pH of the habitat the plant analyses were averaged in five groups. In the first group those plants were placed whose soils had a pH value up to 3—4; the soils of plants classified in the second group were of 4—5 pH, and so on. The 57 samples are rather evenly distributed between 3.86 and 7.23 pH representing the group averages. The only significant correlation — the one between the pH value of the soil and the Mn content of the plant — is shown in Fig. 1.

The analysis of the plant samples — irrespective of a certain degree of heterogeneity in the type of the soils (there were some meadow soils among the forest soils) — revealed tendencies in three regions which later can be taken in consideration in the relationship of nutrient supply, green mass and active agent quantity. So the samples originating from South-Transdanubia contained low quantities of potassium, manganese, zinc and copper, at the same time their molybdenum content and iron/manganese ratio were higher than the average. On the other hand, the samples of West- and North-Transdanubia showed a remarkably high manganese content and low iron/manganese ratio. In plants originating from the Northern Medium-Height Mountains the iron, boron and zinc contents were relatively high.

Table 3

Mean values of microelements in the above-ground parts of Vinca minor as grouped by the chemical reaction of the soil

Average pH in KCl	Number of samples	Fe	Mn	Zn	B	Cu	Fe/Mn
		ppm					
3.86	12	314	302	40.5	37.9	11.1	1.04
4.33	10	227	263	37.4	37.4	12.2	0.87
5.48	10	342	186	40.6	37.2	12.2	1.84
6.53	11	301	98	36.5	40.3	11.9	3.08
7.23	14	370	88	35.7	37.7	11.4	4.22

In the case of zinc, boron and copper the average microelement content of the plant corresponds to the frequency maxima (Table 4). In the case of iron and manganese the distribution curve is not perfectly symmetrical, but extends toward the higher concentrations. So the values most frequently occurring with these two elements are some 40 per cent lower than those averaged on the basis of all samples.

Table 4

Average and most frequent microelement contents in the above-ground parts of Vinca minor

The average of the 57 element samples and the extreme values in ppm			The most frequent concentrations expressed in the number and percentage of samples between the limit values		
Iron	314	139—860	200—250 ppm	16 samples	28%
Manganese	177	46—535	100—150 ppm	17 samples	30%
Zinc	38	19— 64	35— 40 ppm	17 samples	30%
Boron	38	21— 54	35— 40 ppm	15 samples	26%
Copper	11.5	6.4—20.9	10— 12 ppm	16 samples	28%

Changes in the edafic factors acting on *Vinca minor* influence the uptake of certain elements in the same, while that of other elements in the opposite way. E.g. in our material the manganese and zinc contents of the 57 samples showed parallel changes. The 0.38 value of Spearman's rank correlation coefficient is significant at P = 0.01. On the other hand, in spite of the wide range of habitats no iron–manganese antagonism was found ($r_{\text{rank}} = 0.083$), nor cold a copper–boron correlation observed by some be ($r_{\text{rank}} = 0.040$) pointed out.

Discussion

Our investigations into the nutrient and habitat requirements of *Vinca minor* L. have revealed the cause hindering the successful field production of this important medicinal plant. *Vinca minor* differs from most cultivated plants by its high capacity of manganese uptake. This capacity is, in turn, parallel to an increased demand for manganese proved by the fact that the large populations of *Vinca minor* are found on acidic forest soils rich in manganese. In spite of some subspontaneous occurrence encountered in our material, the average pH value measured in potassium chloride was 5.53, i.e. much lower than that determined by MÁTHÉ (1968) for the optimum yield of active agent. In agreement with Máthé's opinion we also find the parameters of optimum life conditions to be different from the environmental parameters of the maximum active agent content. This shade-loving plant when planted to open sunny fields produces more active agent. The mesoclimate of the open fields, on the other hand, with their easily warmed, drier, better ventilated soils ensures a lower mobility for the manganese. According to observations made abroad and in Hungary (TÖLGYESI 1969, TÖLGYESI—CSAPODY 1973) the relatively high calcium content, pH and humus stability value of the meadow-type arable soils often result in a lower mobility of the zinc.

The manganese content which is connected with the chemical reaction of the soil cannot be neglected for phytopathological reasons either. Plants rich in manganese generally have lower protein and water contents, their habitus is firmer and their collenchyma rougher (TÖLGYESI 1969). Plants with a higher manganese content contain more raw fibres (TÖLGYESI 1970). Many data and correlations suggest that the resistance to fungi increases with the manganese content. TÖLGYESI (1965) found a linear correlation between the decomposition rate of the forest litter and the copper content. On the basis of a copper—manganese antagonism pointed out later (TÖLGYESI 1970) this means that the enzymatic decomposition by microorganisms of plants with higher manganese contents takes place more slowly than that of the plants poor in manganese. This opinion is indirectly confirmed by the experiments of NAGY *et al.* (1970) who found the cellulolytic activity to be higher in the soil of plants affected by *Phoma* than in forest soils where resistant plants grew. Without reciting all possible phytopathological consequences of manganese deficiency we mention here that the resistance to fungal diseases of the manganophilous (and at the same time more or less acidophilous) plant species decreases on soils abounding in calcium. E.g. the infection by *Fomes annosus* (Fr.) Cooke in pines is typically such a problem of habitat (WENZEL—KREUTZER 1971). To mention Hungarian examples: in the ecological demands of *Pinus silvestris* and *Pinus nigra* it is the difference between their manganese requirements that is first of all important, and the deterioration of *Pinus silvestris* on lime soils is not only the consequence of a deficient nutrient supply but shows a reduced resistance too. Again, the "potassium demand" and "lime abhorrence" of the sweet chestnut conceals a definite manganese requirement (TÖLGYESI—CSAPODY 1973).

In our opinion the acidophilous and manganophilous biochemical habitus of *Vinca minor* L. was left out of consideration when it was taken under cultivation. The increase in the active agent production under the influence of the insolation and the meadow type of soil is regarded — in agreement with Máthé — as the response of the plant to the unfavourable ecological conditions.

The question is whether we can do anything to prevent the deterioration of *Vinca minor* stands grown in the field. The investigations have revealed that it is not in the absolute active agent content of the soil but first of all in the lower concentration of elements not readily available with an alkaline reaction that the problem lies. It is unfavourable for a definitely acidophilous and manganophilous plant. In shifting the chemical reaction of the soil toward acidity ammonium sulphate and ammoniumdihydrogen phosphate can be taken into account in the first place. These fertilizer types are, however, difficult to purchase, as in Hungary — except for some ornamental cultures — few scientific and practical analyses have dealt with soil amelioration aimed at attaining an acid reaction. In the case of manganese and zinc deficiency appearing as a defect of composition ammonium sulphate in a quantity corresponding to the traditional ammonium nitrate has been applied by TÖLGYESI (1970, 1972) more or less successfully (e.g. in a culture pot experiment on chernozem soil from Mezőhegyes it did not produce demonstrable differences even when applied at a rate of 150 kg N/ha — naturally with optimum water supply —, while at Felsővány, on a calcareous slightly humous sand, plants changing in habitus and composition could be harvested already in the first year. Acidification increased the amount of manganese, zinc and raw fibre.)

For experimental purposes the application of manganic and copper salt solutions, possibly without an additional work phase, in combination with plant protectives or herbicides can also be taken into account.

Acknowledgements

The authors are indebted to Mr. P. Tétényi, director, for calling attention to the subject and providing for undisturbed conditions during the investigations; to the Kőbánya Pharmaceutical Works for the financial support given to the experiments; to Mr. J. Bánkuti, Mr. T. Major and Mrs. J. Varga for their expert assistance.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 79—87 (1976)

TWO NEW NATURAL HOSTS OF TURNIP MOSAIC VIRUS IN HUNGARY

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The present paper gives an account of the spontaneous occurrence of turnip mosaic virus (TuMV, syn.: cabbage black ring virus, */* : */* : E : E : S/Ap) in turnip *(Brassica rapa* L. var. rapa) and cabbage (*Brassica oleracea* L. var. *capitata*) in Hungary. The virus was identified on the basis of test plant reaction and host range, serology, electron microscopy, phid transmissibility, inclusion bodies and physical properties. The two investigated turnip mosaic virus isolates (*HS* and *K30*) seem to be similar in host reactions to TuMV-JN and TuMV-A11 reported from cauliflower and garlic mustard in Hungary (Horváth *et al.* 1975). Electron microscopy revealed flexuous filamentous particles averaging 730 nm in length. The virus was readily transmitted by *Myzus persicae* Sulz. in a non-persistent manner. Both *HS* and *K30* isolates of turnip mosaic virus produced granular cytoplasmic *X*-bodies of oval or irregular shape, often containing crystalline needles. They had the following physical properties: thermal inactivation point 56—58 °C, dilution end point 2×10^{-3}—2×10^{-4}, longevity *in vitro* 2—3 days. The two virus isolates were serologically related to the Yugoslav strain of turnip mosaic virus isolated from cabbage.

Introduction

The first results of experiments with turnip mosaic virus are linked with the name of Schultz (1921). According to the investigations made during the past half century the turnip mosaic virus is spread all over the world and has numerous natural and artificial host plants (Smith 1935, Tompkins 1937, Tompkins *et al.* 1938, Larson—Walker 1939, Walker *et al.* 1945, Broadbent 1957, Uschdraweit—Valentin 1957, Bhargava—Yoshii 1960, Arnold—Bald 1960, Štefanac—Udjbinac *et al.* 1963, Shukla—Schmelzer 1970, 1972, 1973, Feldman—Gracia 1972, Weathers *et al.* 1972, Pontis 1973, Schmelzer—Schmelzer 1974). In the course of studies in cruciferous plants in Hungary the occurrence of turnip mosaic virus has just recently been experimentally proved (Horváth *et al.* 1975) natural hosts being cauliflower *(Brassica oleracea* L. var. *botrytis* (L.) Alef.) and garlic mustard *(Alliaria petiolata* (M. B.) Cavara et Grande). During our further investigations into the distribution of the turnip mosaic virus in Hungary we found two additional natural host plants, i.e. cabbage and turnip. In this paper identification of virus isolates from these two plants is reported.

Material and Method

In the autumn of 1973 among the turnip plants *(Brassica rapa* L. var. *rapa)* breed in the experimental field of the Research Institute for Beet Growing at Sopronhorpács (Hungary) we found some which with respect to symptoms differed from those plants from which radish mosaic virus and turnip yellow mosaic virus had earlier been isolated in Hungary (MAMULA *et al.* 1972, HORVÁTH *et al.* 1973, JURETIĆ *et al.* 1973). The diseased turnip plants were characterized by systemic vein clearing, vein yellowing, severe mosaic with light and dark green patches or blisters and severe distortion and stunting (Fig. 1A). In the same autumn, at the Variety Testing Station at Tordas (Hungary) our attention was attracted by cabbage plants *(Brassica oleracea* L. var. *capitata)* showing typical greyish brown necrotic ring-shaped spots. These spots were particularly conspicuous when looked at from the abaxial part of the leaf (Fig. 1B). From the leaves of the diseased turnip and cabbage plants separate samples were collected. The sample collected from turnip plants was marked with the symbol *HS*, while that taken from cabbage plants with *K30*.

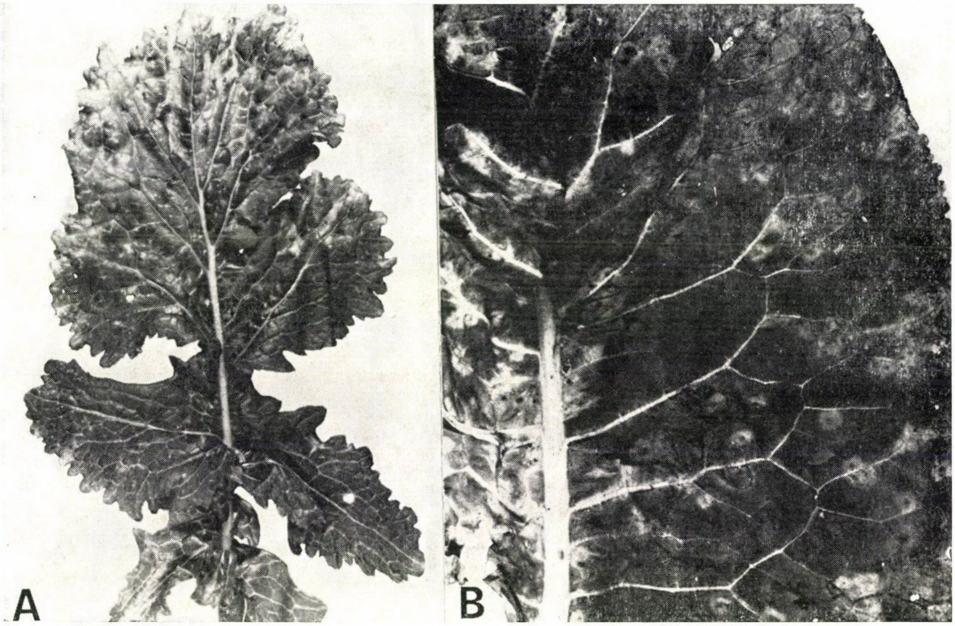

Fig. 1. Symptoms of the spontaneously infected *Brassica rapa* L. var. *rapa* (A) and *Brassica oleracea* L. var. *capitata* p ants (B) with turnip mosaic virus

The two isolates *(HS* and *K30)* were transmitted from turnip and cabbage, respectively, to *Brassica rapa* var. *rapa* and then to other test plants by the conventional technique of grinding young leaves in 0.15 *M* phosphate buffer at *pH* 7.0. In serological experiments immune sera were used against radish mosaic virus, turnip yellow mosaic virus and turnip mosaic virus. The tests performed by the first two sera were done by means of the double diffusion technique (VAN REGENMORTEL 1966, WETTER—LUISONI 1969, MATTHEWS 1970). Slide precipitin tests were used in case of turnip mosaic virus antiserum application. All sera were kindly supplied by the Institute of Botany, University of Zagreb (Yugoslavia). Infected plant sap of turnip was examined by the dipping-method with a Siemens Elmiskop I.

For the aphid transmission studies of *HS* and *K30* isolates, fasted, non-viruliferous aphids were used. *Myzus persicae* Sulz. aphids, starved for 3 hours in glass tubes, were allowed to feed on diseased turnip leaves for 5—10 minutes, and then ten of them were transferred to each single healthy turnip plant. In the transmission studies 30 turnip plants were used for each of the *HS* and *K30* isolates.

The light microscope examinations of inclusion bodies were only performed with living cells of turnip plants. The inclusion bodies were studied in tissue sections taken from the midribs of the turnip leaf. Only epidermis cells were investigated. The physical properties (thermal inactivation point, longevity *in vitro*, dilution end point) of the two isolates were determined in the sap of the infected leaf material of the turnip as source. *Nicotiana tabacum* L. cv. *Xanthi*-nc and *Chenopodium amaranticolor* Coste et Reyn. were used as test plants (Horváth et al. 1975).

Results

The experiments aimed at establishing the host range of the *HS* and *K30* isolates included 35 species of eight plant families. Generally, test plant reactions to isolates *HS* and *K30* corresponded to those provoked by turnip mosaic virus (Table 1, Figs 2 and 3). These data suggested that our isolates could belong to turnip mosaic virus. The symptoms produced by isolates *HS* and *K30* were similar to each other in all plants with the exception of *Brassica*

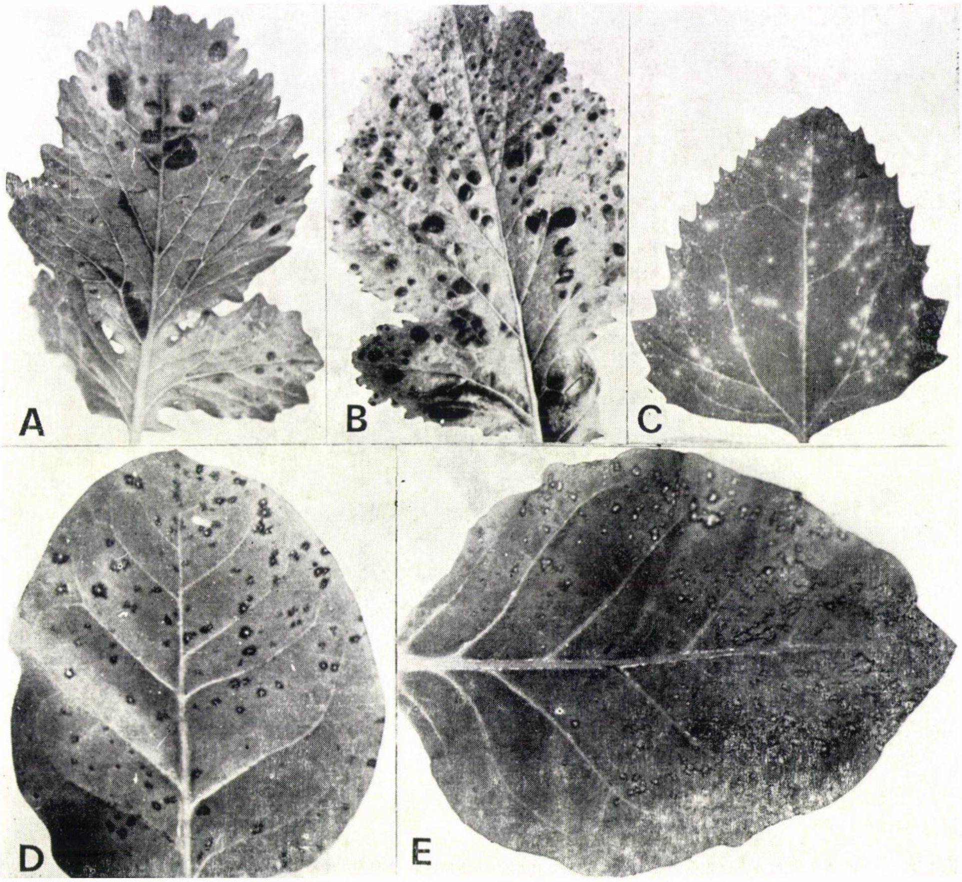

Fig. 2. Symptoms of turnip mosaic virus on various test plants. A and B: Systemic symptoms of the *K30* isolate; C, D and E: Local symptoms of the *K30* isolate (C and D), and *HS* isolate (E). A and B: *Brassica rapa* L. var. *rapa*, C: *Chenopodium amaranticolor* Coste et Reyn., D: *Nicotiana tabacum* L. cv. *Samsun*, E: *Nicotiana tabacum* L. cv. *Xanthi*-nc

oleracea varieties and *Nicotiana glutinosa* L. (Table 1). In the last two plants isolate *K30* provoked much stronger symptoms than isolate *HS*. The plants that were resistant to the two isolates during the host range investigations had been also resistant to two isolates of the turnip mosaic virus described by Horváth *et al.* (1975). Our supposition based on test plant reactions that the *HS* and *K30* isolates belong to the turnip mosaic virus was later unambiguously confirmed by serological reactions; in all serological tests both *HS* and *K30* isolates reacted positively with immune serum of turnip mosaic virus (homologous titer 1/256). These experiments showed, moreover, that isolates *HS* and *K30* were serologically related to a Yugoslavian isolate of this virus which was isolated from cabbage (Miličić *et al.* 1958, 1963). The lack of positive reaction between the two isolates and antisera against radish mosaic and turnip yellow mosaic viruses showed that isolates *HS* and *K30* were free from these viruses.

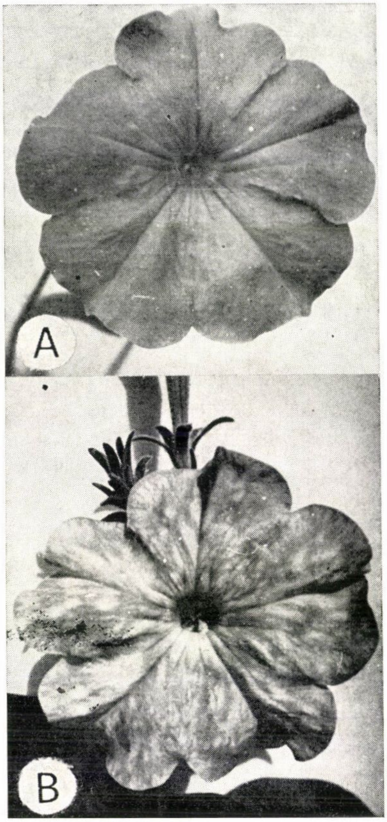

Fig. 3. Healthy flower of *Petunia hybrida* hort. ex Vilm. (A) and colour flower breaking (B) of the diseased plant inoculated with the *K30* isolate of turnip mosaic virus

Table 1

*Reaction of several plants to the HS and K30 isolates of turnip mosaic virus**

AIZOACEAE	
Tetragonia echinata Ait.	I: Chlorotic spots II: Not infected
T. tetragonoides (Pall.) O. Ktze	I: Chlorotic spots II: Not infected (sometimes secondary spots on the non-inoculated leaves)
AMARANTHACEAE	
Gomphrena globosa L.	I: Gray local lesions with pink border II: Not infected
CHENOPODIACEAE	
Atriplex nitens Schk.	I: Local chlorotic or necrotic spots II: Sometimes secondary spots on the non-inoculated leaves
Chenopodium amaranticolor Coste et Reyn.	I: Chlorotic local lesions that turn into necrotic lesions (Fig. 2C) II: Not infected
Ch. foliosum Aschers.	I: Chlorotic and necrotic lesions II: Not infected
Ch. murale L.	I: Minute necrotic lesions II: Not infected
Ch. quinoa Willd.	I: Chlorotic local lesions II: Not infected
Obione sibirica (L.) Fisch.	I: Chlorotic local lesions II: Not infected
CRUCIFERAE	
Brassica campestris L.	I: Not infected II: Systemic vein clearing, mosaic with light and dark green patches or blisters
Br. carinata A. Br.	I: Not infected II: Systemic vein clearing and mosaic
Br. oleracea L. var. *capitata*	I: Not infected II: Isolate *K30;* pale green rings, mottling, black necrotic rings Isolate *HS;* weak pale green rings and mottling
Br. oleracea L. var. *botrytis*	I: Not infected II: Isolate *K30;* pale green rings, mottling, black necrotic rings Isolate *HS;* weak pale green rings and mottling
Br. rapa L. var. *rapa*	I: Chlorotic and sometimes necrotic local lesions II: Systemic vein clearing and veinal flecking, developing into severe mosaic with light and dark islands Severe distortion and stunting (Fig. 2A and B)
Bunias orientalis L.	I: Not infected II: Systemic mosaic spots
Cherianthus cheiri L.	I: Not infected II: Systemic leaf distortion, mottling and colour breaking of flower
Lunaria annua L.	I: Not infected II: Mosaic and leaf deformations
Matthiola incana (L.) R. Br.	I: Not infected II: Mild mottling

* I, denotes rubbed leaves (local symptoms); II, denotes leaves developed after inoculation (systemic symptoms)

Table 1

(continued)

CUCURBITACEAE	
Bryonia alba L.	I: Not infected II: Not infected
B. dioica Jacq.	I: Not infected II: Not infected
Cucumis sativus L.	I: Not infected II: Not infected
Cucurbita pepo L. convar. *patissonina* Greb. f. *radiata* Nois.	I: Not infected II: Not infected
LEGUMINOSAE	
Phaseolus vulgaris L. cv. Red Kidney	I: Not infected II: Not infected
SOLANACEAE	
Capsicum annuum L.	I: Not infected II: Not infected
Datura stramonium L.	I: Not infected II: Not infected
Nicotiana chinensis Fisch.	I: Local chlorotic spots II: Not infected
N. clevelandi A. Gray	I: Necrotic local lesions II: Necrosis on top leaves
N. glutinosa L.	I: Not infected II: Isolate *K30;* conspicuous systemic mottling and leaf distortion Isolate *HS;* systemic mottling and leaf distortion (sometimes erratic reaction)
N. megalosiphon Heurck. Muell.	I: Not infected II: Systemic mottling
N. occidentalis Wheeler	I: Local necrotic lesions II: Systemic mosaic, sometimes necrotic spots
N. tabacum L. cv. Bel 61—10, Samusn, White Burley, and Xanthi-nc	I: Local necrotic lesions (Fig. 2D—E) II: Not infected
Petunai hybrida hort. ex Vilm.	I: Black necrotic local lesions II: Systemic vein clearing, mottling and colour breaking of flower (Fig. 3)
Solanum capsicastrum Lk.	I: Not infected II: Not infected
S. ochroleucum Bast.	I: Not infected II: Not infected
UMBELLIFERAE	
Ammi majus L.	I: Not infected II: Not infected
A. visnaga (L.) Lam.	I: Not infected II: Not infected

Electron microscope analysis of the sap of infected turnip plants revealed that our isolates had flexuous filamentous particles of about 730 nm length. It must be pointed out that only several particles were measured. The *HS* and *K30* isolates were easily transmitted by *Myzus persicae* aphids from one turnip plant to another in a non-persistent manner. Investigations concerning the cell inclusion bodies showed that *K30* and *HS* isolates produced *X*-bodies in the cytoplasm of infected plants. The bodies were oval or of irregular shape, with a granular structure (Fig. 4). At a later stage of the infection numerous minute crystalline needles were usually seen in the *X*-bodies. The *X*-bodies of our isolates were in every respect similar to those described for turnip mosaic virus by Rubio (1956), Miličić (1956), Miličić *et al.* (1963) and Stefanać—Miličić (1965). Inclusion bodies were observed in the cells of *Brassica rapa* var. *rapa*, *Nicotiana glutinosa*, *N. megalosiphon* Heurck. et Muell. and *Petunia hybrida* hort. ex Villm. When examining the physical properties of the two isolates we found that they were slightly different from each other (see Table

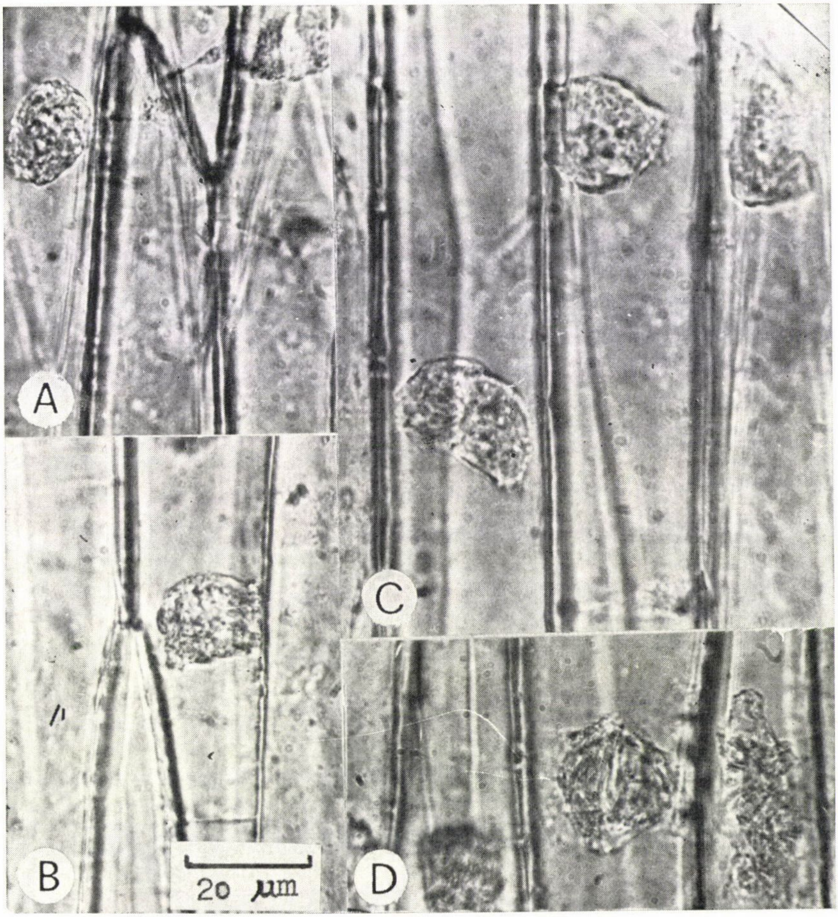

Fig. 4. Inclusion bodies (*X*-bodies) in epidermal cells of *Brassica rapa* L. var. *rapa* leaf (midrib region) inoculated with the *K30* isolate of turnip mosaic virus

2). Additionally, *HS* and *K30* isolates differed also slightly in physical properties from two isolates of turnip mosaic virus found earlier in Hungary (Horváth *et al.* 1975).

Table 2

Physical properties of the HS and K30 isolates of turnip mosaic virus

Isolates of turnip mosaic virus	Physical properties*		
	TIP (in °C)	DEP	*Liv* (in days)
HS	58	$10^{-3}-2\times10^{-4}$	3
K30	56	2×10^{-3}	2

* TIP, thermal inactivation point; DEP, dilution end point; *Liv*, longevity *in vitro*

On the basis of differences in physical properties, and especially with respect to the different reaction of *Brassica oleracea* varieties and *Nicotiana glutinosa* to isolate *HS* and *K30*, it is likely that the two isolates belong to different strains of turnip mosaic virus. Accordingly, isolate *HS* should be attached to the *ordinary strain*, and *K30* isolate to the *cabbage strain* of this virus (comp. Yoshii 1963). In this respect isolate *HS* is similar to the TuMV-All isolate, and *K30* to the TuMV-JN isolate of turnip mosaic virus found earlier in Hungary (Horváth *et al.* 1975).

Acknowledgements

We are indebted to Dr. N. Ljubešić for the electron microscope examinations, and to Miss K. Molnár for her assistance in the experiments.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 89—97 (1976)

EPIDERMIS STUDIES ON TOBACCO VARIETIES

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Due to its close contact with the outer world the epidermis is greatly influenced by the environment. All important parameters of the epidermis give definite responses to the environmental effects, first of all to temperature. The examined parameters were: 1. Number of stomata, 2. Size (length and width) of the stoma, 3. Shape of the stoma (length) (width), 4. Stomal ratio, 5. Number of epidermis cells, 6. Size (length and width) of the epidermis cells, 7. Shape of the epidermis cells (length/width), 8. Stoma index. Changes occurring in the specific anatomical characters are conspicuous and show the same tendency. The five varieties included in the investigations were: 1. "Szabolcsi Sota", 2. "Kerti", 3. "Hevesi Delcrest", 4. "Burley B 21", 5. "Burley B II." They were sown at the following dates: 21 and 31 March, 7, 14 and 21 April 1973. The number of stomata both on the adaxial and abaxial surface of the leaf were found to increase. The stomata changed their shapes to a smaller, the epidermis cells to a greater extent; the length of the latter decreased in direct ratio to late sowing, that is, the epidermis cells became ellyptic. Changes in the ratio of stomata as a function of sowing time were conspicuous in all varieties. It was in this parameter that the greatest differences were found between the varieties. Differences in the stomal index according to variety and sowing time were less marked and the tendency of changes was uniform. The evidence of the examinations show a distribution according to the optimum curve.

Introduction

The environmental factors act on the whole plant, as shown by many signs. The question arose whether their effects could be observed in the qualitative and quantitative changes of the tissue structure of plants too. Our investigations were thus aimed at registering the changes occurring in the epidermis as a tissue in direct contact with the environment.

Changes caused by the xeromorph and hygromorph conditions in the epidermis have been dealt with in a number of text-books (Nosatovsky 1951, Szalai 1968, Hortobágyi 1968). Epidermis changes caused by ecological factors are discussed in papers by Frenyó (1969) and Simon-Wolcsánszky—Molnáros (1964). The question was raised whether only an intensive environmental influence caused changes, or minor ones too could have an effect. No data concerning this question are available in the literature. The presented data give account of difference in the epidermis structure of leaves originating from different growing sites.

These differences can only be explained after a study on the gradient of changes in the factors.

An ecological series obtained by fractional sowing is suitable for this purpose. The correctness of this method has been proved for a number of plants in connection with other characters e.g. phenological phenomena, yield elements, value of components (MÁNDY 1969).

In the ecological series the distribution follows the optimum curve. The question arises whether the same statement can be made for the quantitative properties of the epidermis as well.

Material and Method

The examined varieties whose seeds were placed at our disposal by the Seed Unit of the Administration of the Tobacco Monopoly were: 1. "Szabolcsi Sota", 2. "Kerti", 3. "Hevesi Delcrest", 4. "Burley B 21", 5. "Burley B II".

In studying the ecological factors an ecological series developed by Mándy's fractional sowing method was used.

The five sowing times were: A: 21 March 1973, B: 31 March 1973, C: 7 April 1973, D: 14 April 1973, E: 21 April 1973. The seeds were sown in crates, and the plants raised in a platic tent without soil heating. The regular irrigation of plants was ensured. The epidermis of primary foliage leaves was the object of investigations. The leaves were collected at the age of 50 days and the collodium impression method applied. Samples were taken from the adaxial and abaxial surfaces of the primary foliage leaves of five plants per treatment and replication in all varieties. Five cells or five stomata, respectively, of each sample were measured. The data of the tables and diagrams are the averages of these, altogether 125, measurements.

Of the characteristics quantitatively measurable by microscope examinations the following are considered as important by SÁRKÁNY—RIEDL (1951, 1952): 1. Number of stomata, 2. Size (length and width) of the stoma, 3. Shape of the stoma (ratio of length and width), 4. Stomal ratio, 5. Epidermis cells, 6. Size (length and width) of the epidermis cells, 7. Shape of the epidermis cells (length/width), 8. Stomal index.

The average air temperature and the number of sunshine hours during the 50 days from sowing to the collection of the investigation material are shown in Table 1.

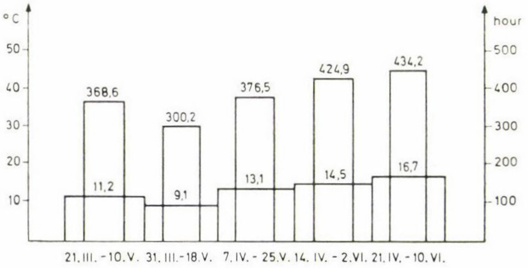

Fig. 1. Average air temperature and number of sunshine hours in 50 days. The left-side vertical axis shows the temperature, the right-side one the number of sunshine hours. On the horizontal axis the wide columns indicate the temperature values corresponding to the 50 days interval, and the narrow columns in them show the number of sunshine hours

Results

As a result of the environmental effects the following could be observed on the epidermis of the primary foliage leaves:

1. *Number of stomata.* In all varieties great changes were found in the number of stomata. On the upper surface of the variety "Szabolcsi Sota" a minimum value of 40 stomata/mm^2 was found in the stand sown on 14th April. The first three sowing times showed no considerable change in the number of stomata. The number of stomata was the highest in the stand sown on 21st April: 58/mm^2. The changes showed nearly the same trend in the other varieties too. With the first four sowing times — between 24th March and 14th April — the stoma numbers obtained ranged from 33 to 50. The latest sowing resulted in outstanding values in all varieties. Similar tendency was seen on the lower surface of the leaf too, with the difference that here the number of stomata per unit area was larger. The minimum value — 38 stomata/mm^2 — was obtained with the first sowing time in the variety "Hevesi Delcrest". The maximum value — 101 stomata/mm^2 — was shown by the variety "Szabolcsi Sota" when sown on 21st April. The maxima of all varieties were found in the stands sown at this date.

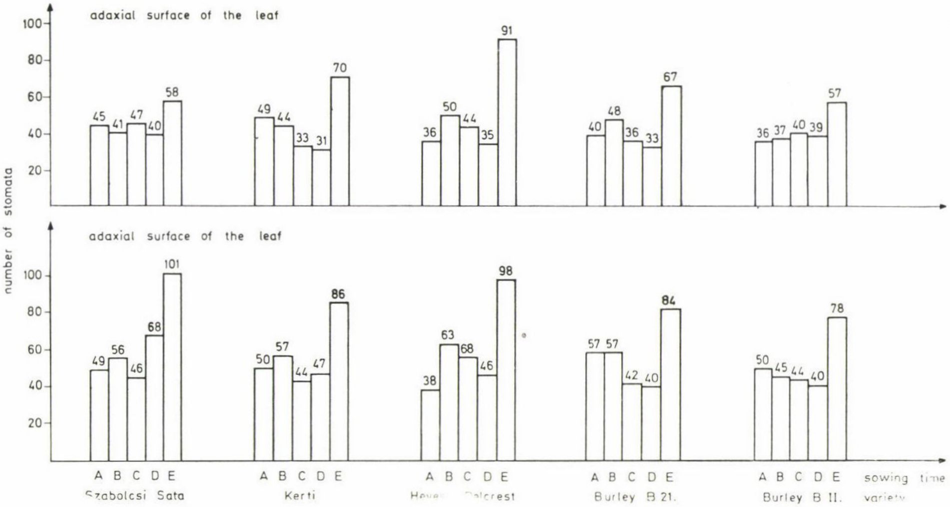

Fig. 2. Stoma numbers of primary foliage leaves of tobacco per mm^2. On the vertical axis the number of stomata per mm^2, on the horizontal axis the values obtained on the upper and lower leaf surfaces with the different sowing times are shown

2. *Size of the stoma.* This can be illustrated by giving the lengths and widths. On the upper surface of the leaf the lengths range from 19 to 32 μm, and the widths from 12.5 to 17.6 μm. On the lower surface of the leaf the lengths of the stomata were between 25.3 and 28.81 μm, and the widths between 13.5 and 17.1 μm. In the varieties "Szabolcsi Sota" and "Kerti" the trend of change was similar: when sown on 14th and 21st April they had smaller stomata, while in the variety "Hevesi Delcrest" and the two "Burley" varieties

Table 1

Size of stomata on the primary foliage leaves of tobacco (μm)

Variety	Szabolcsi Sota		Kerti		Hevesi Delcrest		Burley B 21		Burley B II	
Sowing time	length	width	length	width	length	width	length	width	length	width
	millimicron									
A. adaxial	27.2	15.2	28.5	14.4	24.3	13.7	23.0	12.9	23.8	15.6
abaxial	24.6	13.6	24.8	14.1	24.9	12.9	25.4	14.0	23.5	14.0
B adaxial	25.8	14.3	28.0	14.2	32.0	17.6	26.6	14.4	25.8	14.3
abaxial	23.6	14.5	25.6	14.4	28.8	15.7	27.6	15.6	27.8	15.0
C. adaxial	27.4	14.9	28.5	16.2	23.7	14.9	23.4	13.8	22.4	13.7
abaxial	25.0	14.2	25.3	17.2	24.1	13.5	23.9	12.9	22.2	12.8
D. adaxial	22.8	12.8	25.2	12.5	25.8	13.9	26.1	14.2	25.4	13.3
abaxial	24.6	13.9	24.8	13.4	28.5	14.9	24.9	14.0	22.3	13.7
E. adaxial	20.3	16.6	21.6	13.9	22.7	13.3	21.1	13.0	22.1	14.1
abaxial	20.6	12.8	20.6	11.7	19.8	11.5	21.9	12.7	25.3	13.5

Table 2

Shape of stomata on the primary foliage leaves of tobacco

Variety	Szabolcsi Sota	Kerti	Hevesi Delcrest	Burley B 21	Burley B II
Sowing time	length/ width	length/ width	length/ width	length/ width	length/ width
A. adaxial	1.78	1.97	1.77	1.85	1.52
abaxial	1.87	1.75	1.91	1.81	1.67
B. adaxial	1.97	1.96	1.82	1.13	1.79
abaxial	1.63	1.77	1.83	1.76	1.85
C. adaxial	1.77	1.84	1.76	1.58	1.69
abaxial	1.76	1.47	1.70	1.84	1.73
D. adaxial	1.78	2.01	1.83	1.83	1.61
abaxial	1.76	1.08	1.91	1.77	1.61
E. adaxial	1.20	1.50	1.60	1.61	1.57
abaxial	1.60	1.76	1.71	1.88	1.87

the stomata were longer with the sowing time of 31 March. The optimum curves here showed remarkable peaks.

3. *Shape of the stoma.* It can be characterized with the ratio of length/width on the basis of Sárkány—Riedl's table. Accordingly, the stomata are longish in all varieties, as the ratio is higher than 1.3; in the variety "Kerti" — when sown on 14th April — it was as high as 2.01, that is the stomata were extremely elongated. Two exceptions were found: in the variety "Burley B 21", when sown on 31st March, and in the "Szabolcsi Sota", when sown on 21st March the ratio was below 1.3, that is, the stomata were ellyptic.

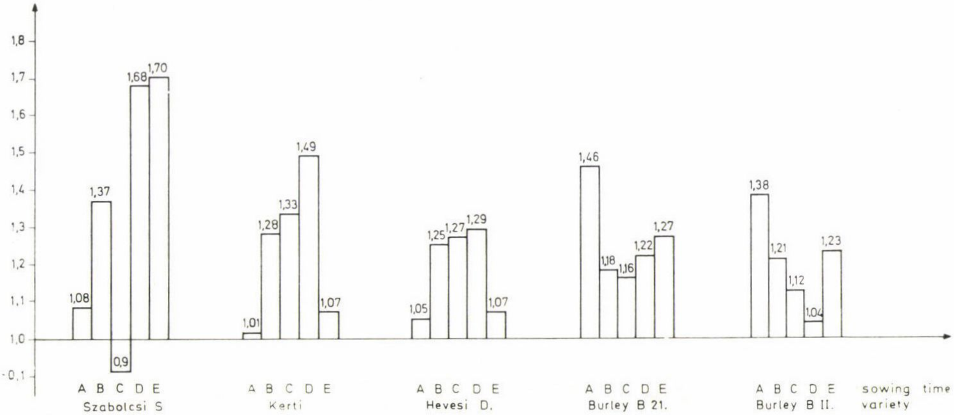

Fig. 3. Stoma ratio on primary tobacco foliage leaves. The vertical axis shows the ratio of the number of stomata per mm^2 on the lower leaf surface to that on the upper leaf surface. On the horizontal axis the values of the varieties obtained with the different sowing times are indicated

4. *Stoma ratio.* It is an interesting characteristic of the epidermis. It expresses the ratio of stoma number on the lower to that on the upper surface of the leaf. According to the evidence of the numerical data it is a rather constant value in the case of tobacco. Apart from an only exception there are more stomata on the abaxial surface of the leaf. In the variety "Szabolcsi Sota" in three treatments more stomata were found on the adaxial surface; when sown on 14th April, on the other hand, this variety showed the greatest difference in favour of the abaxial surface.

Apart from the fact that the number of stomata was higher on the lower surface of the leaf, the different time of sowing still caused deviations: the varieties "Kerti" and "Hevesi Delcrest" showed a nearly equal number of stomata on the upper and lower surfaces of the leaf when sown on 21st March and 21st April, while in the two "Burley" varieties the greatest differences were found in these cases. In these varieties the number of stomata on the upper and lower surface of the leaf were nearest in the 7 April and 14 April treatments.

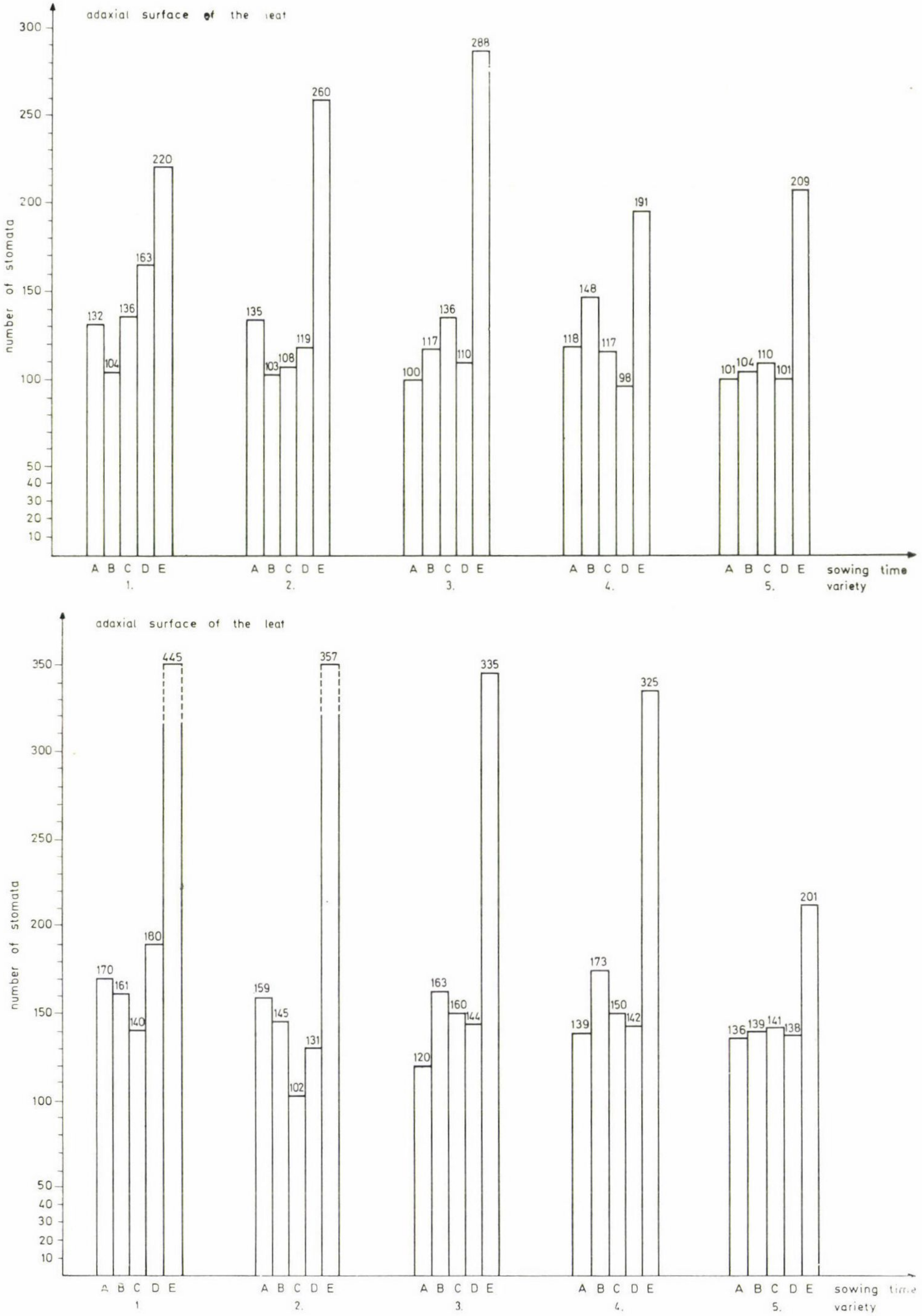

Fig. 4a. Number of epidermis cells on the upper leaf surface of primary tobacco foliage leaves. *b)* Number of epidermis cells on the lower surface of primary tobacco foliage leaves (per mm²). The vertical axis shows the number of cells per mm², the horizontal axis the values obtained with the different sowing times

Table 3

Size of epidermis cells on the primary foliage leaves of tobacco (μm)

Variety		Szabolcsi Sota		Kerti		Hevesi Delcrest		Burley B 21		Burley B II	
Sowing time		length	width	length	width	length	width	length	width	length	width
		millimicron									
A.	adaxial	95.8	33.8	101.2	35.9	104.7	37.4	103.6	35.3	107.5	36.6
	abaxial	84.4	31.2	95.9	30.81	109.8	37.5	103.8	35.3	81.5	34.9
B.	adaxial	102.6	38.9	100.9	37.9	96.7	34.9	83.4	30.2	103.2	35.3
	abaxial	84.4	33.0	92.5	37.3	94.3	30.2	86.3	39.7	99.7	34.2
C.	adaxial	84.2	46.8	108.8	57.8	78.9	46.0	87.9	40.5	95.6	45.9
	abaxial	89.2	38.2	102.6	39.7	97.8	31.3	93.6	31.7	88.6	33.0
D.	adaxial	84.8	31.9	103.4	37.9	100.9	35.2	104.3	37.7	100.2	56.3
	abaxial	84.5	26.6	104.2	35.3	96.9	34.7	99.7	32.3	104.3	37.7
E.	adaxial	63.0	37.2	53.2	34.2	62.3	37.7	56.6	44.8	70.2	49.7
	abaxial	52.2	21.6	53.3	22.5	56.9	23.9	58.3	27.3	77.1	27.2

Table 4

Shape of epidermis cells on the primary foliage leaves of tobacco

Variety		Szabolcsi Sota	Kerti	Hevesi Delcrest	Burley B 21	Burley B II
Sowing time		length/width	length/width	length/width	length/width	length/width
A.	adaxial	2.83	2.81	2.79	2.92	2.93
	abaxial	2.69	3.12	2.93	2.91	2.33
B.	adaxial	2.63	2.66	2.77	2.75	2.91
	abaxial	2.55	2.46	3.12	2.17	2.91
C.	adaxial	1.80	1.88	1.71	2.17	2.12
	abaxial	2.30	2.57	3.12	2.95	2.68
D.	adaxial	2.65	2.71	2.86	2.76	2.75
	abaxial	3.18	2.95	2.79	3.09	3.47
E.	adaxial	1.70	1.50	1.60	1.25	1.43
	abaxial	2.41	2.42	2.37	2.13	2.83

5. *Number of epidermis cells.* The minimum of all varieties was 89, the maximum 288 epidermis cells per mm^2 of the adaxial surface of the leaf. The tendency of change in the number of cells also follows the optimum curve. The maxima were unsually high in the last treatment sown on 21st April. The number of cells showed a similar trend on the abaxial surface of the leaf.

6. *Size of the epidermis cells.* The longest and shortest cells (108,7 and 53.2 μm, respectively) were found in the variety "Kerti" on the adaxial surface of the leaf. The greatest width — 56,3 μm — was found in "Burley B 21" sown on 21st April, and the smallest width in the same variety when sown on 31st March.

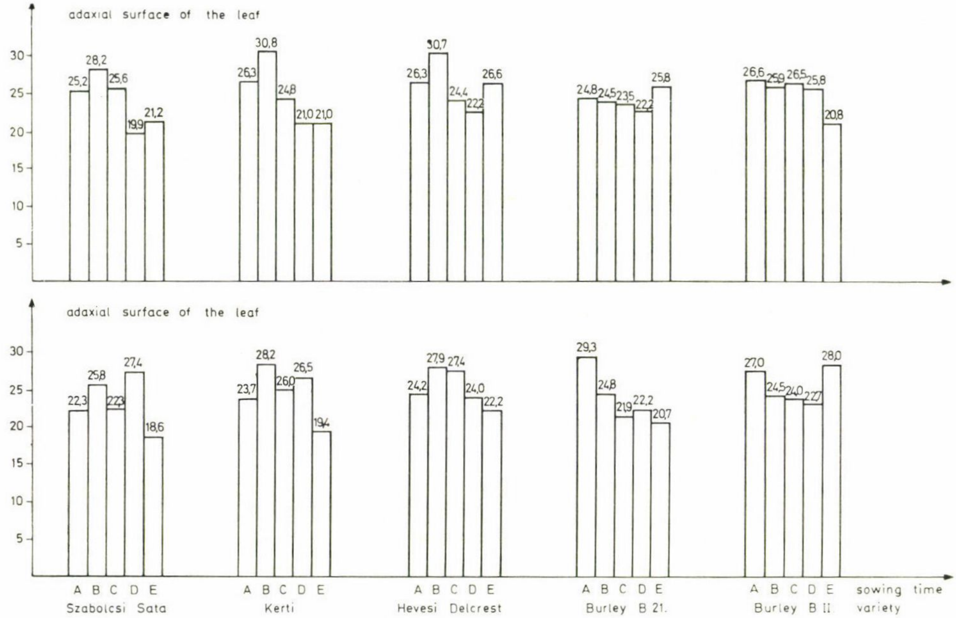

Fig. 5. Stoma index of primary foliage leaves of tobacco on the adaxial and abaxial surface of the leaf. On the vertical axis the index values are found, while the horizontal axis shows the values obtained with the different sowing times

7. *The shape of the epidermis cell.* It can be characterized again by the ratio of the length and width of the cell, which shows the degree of elongation. The higher the ratio, the more elongated the cells. Late sowing remarkably decreased the length of the cells on the upper leaf surface of all varieties.

According to our experiences up to a value of 2.0 the cells are nearly ellyptical, at a ratio of 2.0—2.5 they are elongated and between 2.5 and 3.5 very elongated. There is no considerable difference in the size of cells between the adaxial and abaxial surface of the leaf.

8. *Stoma index.* It expresses the relationship between the number of stomata and that of the epidermis cells per unit area. The index values range between 19 and 31 which — when compared with the corresponding data of the literature — can be considered as balanced. The number of epidermis cells per stoma on the upper leaf surface is the lowest (19.88) in the variety "Szabolcsi Sota", that is, the stomata show the greatest density in this variety. The highest index value in this variety (28.17) was found in the treatments sown in March. It was with the same sowing time that the maximum was attained in the varieties "Kerti" and "Hevesi Delcrest", while the minimum was found in the two latest treatments. The distribution of stomata was the most uniform in the two "Burley" varieties: the index value only ranged from 20 to 26. The values of the lower leaf surface were similar to those obtained for the upper surface of the leaf.

Acknowledgement

Thanks are due in this place too to József Ujhelyi, leading research worker, for showing how to apply a "refined" variation of the mentioned collodium impression method.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 99—126 (1976)

BASIC AMINO ACID-PHOSPHATES OF MYOFIBRIL AND MYOSIN

I. OCCURRENCE OF PHOSPHOHISTIDINE IN MYOSIN AND MYOFIBRIL

By

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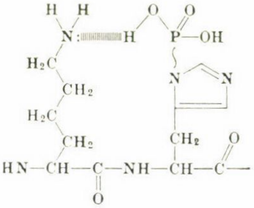
On the basis of our previous work (Fazekas *et al.* 1973, 1974 a, b) we have found that myosin contains a definite quantity — 10—12 g atoms — of phosphorus of covalent bond. In the course of our investigations we isolated 4 M histidine phosphate; in the present paper the circumstances of the isolation are described in detail. Perrie *et al.* (1973) isolated and identified serine phosphate from each of two 18 000 mole weight light chains of myosin, so at present the origin of 6 g. atoms of covalent bond phosphate is solved. In our examinations we found that the histidine phosphate originated from the heavy chain of myosin, its peptides were much more stable than the free phosphohistidine. We assume that further covalent bond phosphates occur in the myosin, the present method is not, however, suitable to isolate them. A phosphopeptide could be produced from myosin or its heavy chain by tryptic digestion too. In a number of cases even more covalent bond phosphates than the above value were measured with a direct method, but a large proportion of them proved to be unstable during the hydrolysis. We tried to bring into harmony the role and biological meaning of histidine phosphates as found by us with the available literary data, and on this basis contribute a much more important role to histidine — and possibly to other phosphates of covalent linkage too — in muscle contraction than that played in the simple Mg^{2+} and Ca^{2+} ATP-ase activity, respectively.

Introduction

The role of phosphorylated proteins in the intracellular metabolism has been known for a long time (Agren *et al.* 1954). Owing to their assumed functions in regulation they have come into the limelight in the muscle as well. Walsh *et al.* (1968) were the first to give account of the occurrence of protein-kinase enzymes catalysing the protein phosphorylation in striated muscle too. In five short years these enzymes were pointed out in both the striated and smooth as well as the heart muscle, and in vitro were found to bind phosphate from the ATP on the casein and histones, on one of the amino acid groups of the protein chain (Walsh *et al.* 1968; Corsin *et al.* 1970, 1973; Zapf—Froesch 1972; Sands *et al.* 1973a, 1973b; Rubin *et al.* 1972). These enzymes have gained a biological meaning by partly exposing their natural substrates occurring in the muscles. Phosphorylation of glycogen phosphorylase (the phosphorylated active, and dephosphorylated inactive form, Cori *et al.* 1943; Krebs *et al.* 1958), glycogen synthetase (Rosenkraus—Larrner 1973), phosphorylation of one of the proteins of the sarcoplasm (Makinose 1972) and one of the proteins of the sarcolemma (SULAKHE *et al.* 1973) as well as of the histone fractions of the muscle cell nuclei are already known.

Keen interest has recently been excited by the fact that the proteins of the myofibrils also become phosphorylated. The phosphorylation of myosin was first supposed by KITAGAVA—TONOMURA (1962), then PERRIE *et al.* (1973) pointed out that the 18 500 molecular weight subunit of the myosin became phosphorylated. As to the other myofibrillar proteins, the phosphorylation of actin was described by PRATJE—HEILMAYER (1972), that of the 37,000 molecular weight T-component and 21,000 molecular weight inhibitor component of troponin was made known by PERRY—COLE (1973, 1974) and STULL *et al.* (1972). In these proteins the phosphate esters, the alcoholic OH group of the serine or treonine are formed. The phosphate esters seem to form an essential part of the active centre of numerous hydrolase type enzymes (MATEJA—DEGENS 1971).



AGREN *et al.* (1951) were the first to notice that in the caseins not only the serine and threonine became phosphorylated but — as pointed out by PERLMAN (1954) and PERLMAN (1955) — some 40 per cent of the phosphate is bound to the alkali-stable and N-phosphate linkages of other amino acids. ZETTERQUIST (1967) found in rat liver enzymes transferring the — phosphate of ATP to histidine. WALINDER *et al.* (1968) isolated lysine and histidine phosphates from bovine liver, while DELUCA *et al.* (1963) histidine phosphate from mitochondrium. Since COLOMB *et al.* (1972) were able to isolate a protein containing basic amino acid phosphate from bovine heart muscles, we found it obvious to assume the presence of basic amino acid phosphate in the myofibrillar proteins of the striated muscle too. A long series of investigations made with a large number of mitochondria as well as with myofibril and myosin showed that myofibril — and myosin within — were the main sources of phosphohistidine directly from myosin.

Material and Method

In the experiments skeletal muscles of four months old normal rabbits were used. Myofibril was prepared from 400 g of the ground material with the method of PERRY—CORSI (1958) and washed in borate buffer until the supernatant showed a value lower than 0.1 E_{280}. The dry substance content of the myofibrillar suspension was 36.8 mg/ml, but its protein content when measured with the microbiuret method of **Goa** (1963) only 27.2 mg/ml. This preparation was freed from lipids as described for myosin, and 8.72 g dried myofibril powder was obtained (39.2 per cent of the initial material).

The myosin was prepared after **Szent-Györgyi** (1947) by the modified technique of **Hasselbach—Schneider** (1951) from 600 g of ground back and leg muscles of a 3—4 months old rabbit.

Both kinds of starting material were treated by the method of **Kleinschmith** *et al.* (1966) in 10 vol. of chloroform–methanol (2 : 1, by vol.) and the precipitates were regained from the organic layer by centrifuge. After cooling in an ice-water bath the precipitates were dissolved in a sufficient volume of ice-cooled 0.1 M NaOH solution by stirring. After some 30 minutes it was warmed to room temperature and stirred occasionally for another hour. The undissolved residues (collagen) were removed by centrifuge and the protein regained from the clear solution, cooled and mixed with two volumes of ice-cooled acetone. (Stirring prevents it from rough precipitation.) After this the precipitate was suspended in ethanol–ether (1 : 1), then in acetone, finally in ether, and air dried. Once we omitted the dissolution of 0.1 M NaOH, and once the total treatment of lipid solvents. We obtained from the 350 ml of concentrated myosin solution 20 g air dried myosin powder and 1.45 g lipid (the latter is equivalent to 36 250 g lipid per 5×10^5 g protein). In other cases from chromatographied myosin (on DEAE-cellulose column) 10.16 and 8.75 g lipid free myosin was obtained, respectively. The ratio of other preparations and the distribution of the phosphorus content are shown in the results.

The air dried preparation was weighed and hydrolysed in 10 ml of 3 M KOH/g protein or 3 M NaOH for 3 hours at 100 °C in a sealed glass bottle by the method of **Walinder** *et al.* (1968). After cooling to room temperature the unhydrolysed precipitates were removed from the myofibril hydrolysate by centrifuge, treated as collagen and purified according to **Fujimoto** (1968) and **Bannister—Burns** (1972); then the supernatant was cooled to 0—4 °C and the pH adjusted to 2.9 with ice-cooled acetic acid (formic acid buffer) and passed through Dowex 50 (H^+ form) column in a cold room. It was 4 cm wide in diameter and higher by 3 cm for each ml of the hydrolysate.

The effluent was collected in a 200 ml Ehrlenmeyer bottle containing 10 ml 1 M NaOH solution, and the pH was maintained above 9.0 with NaOH solution. The effluent was diluted 50 times with distilled water and applied on a 2.2 cm wide and 3 cm long Dowex 1×8 (Cl^- form) column per g protein in a cold room. Elution was performed by the linear or concentration gradient method of chromatography with $KHCO_3$ solution as shown in the figures.

The phosphohistidine was synthesized with the Rathlev—Rosenberg method (**Rathlev—Rosenberg** 1956), but the direct mono-1-phosphohistidine was prepared by Hultquist's method (**Hultquist** 1968). 0.025 M (= 0.42 per cent) histidine and 0.33 M (= 4 per cent) phosphoamidate were mixed in 50 ml water and stirred for an hour at room temperature maintaining the pH at 8.0. After 60 minutes the pH was adjusted above 9.0 with 1 M NaOH, the mixture cooled in an ice-bath and precipitated as calcium salts then stored at —20 °C. The reaction mixture contained a significant quantity of 1,3-diphosphohistidine. 3-Phosphohistidine was prepared from 2 g (0.25 M) histidine and 3 g (= 60 mg/ml) phosphoamidate dissolved in 50 ml water and kept at room temperature for two days while stirred now and then. All synthetic mixtures were chromatographied on Dowex 1×8 (Cl^-) column and the appropriate fractions pooled and precipitated as calcium salts.

The histidine and phosphohistidine were checked in every chromatographied fraction with the Pauly reaction as applied by **DeLuca** *et al.* (1963), and the dried paper chromatograms were sprayed with concentrated acetic acid, kept at 100 °C for 30 minutes then treated with **Ames-Mitchel**'s (1952) spray reagent and finally with 20 per cent Na_2CO_3 solution.

Isolation of the heavy chain from the myosin of the long. dorsii muscle was carried out according to **Perrie** *et al.* (1973). Only fraction IV. of the DEAE-cellulose column chromatographied myosin (**Fazekas** *et al.* 1974b) was used for heavy chain preparation by trypsin digestion (DCG treated, Serva, Heidelberg), 830 mg of heavy chain: 9 mg of activated trypsin was digested on 0.02 M KCl 0.02 M Tris buffer (pH 8.5) for three hours at room temperature, while a further 9 mg of trypsin was treated for a longer time, in a thermostate at 37 °C. The digest was diluted to 200 ml and applied per 5 g on DEAE-cellulose (E_{52}) column equilibrated with 10 mmols Tris-HCl buffer (pH 8.0) and washed with the buffer until the effluent solution showed on OD value lower than 0.1 at 235 nm, then a linear gradient chromatography between 0 and 0.15 M NaCl followed, with a step by step method, as shown in Fig. 13. The main fraction of phosphopeptide was pooled and then dissolved in a small volume of distilled water, gel-filtrated on a 1.8×66 cm (= 170 cm^3) column of Sephadex G-25 gel and eluated with a 0.02 M NH_3 solution.

The phosphopeptides of alkaline and tryptic digests, and fractions of synthetic phosphohistidine were purified and separated on Whatman No. 3 paper. The cold dried samples were dissolved in a small volume of distilled water and applied on paper, formed the line and ran as one dimensional ascending chromatograms. On the left edge of every chromatogram histidine, phosphohistidine or a synthetic mixture of histidine and phosphohistidine run as controls were found. The chromatograms were developed in a mixture of n-propanol–ethanol–water-NH_3 (30 : 30 : 39 : 10 by vol) as described by DeLuca *et al.* (1963). This solution mixture showed the best separated lines of peptides on the chromatogram among the tried running mixtures even when the peptides contained significant quantities of $KHCO_3$. The dried paper chromatograms were checked under UV light and the lines of peptides marked with a pencil, then the left edges of the chromatograms cut off at a width of 12 cm. These stripes were coloured with ninhydrin spray reagent by the method of Heilman *et al.* (1957), and the nonfluorescent peptides marked on the chromatograms. When the histidine content of peptides was ambiguous, the method of Sanger—Tuppy (1953) was used. A sufficient quantity of phosphohistidines appeared as faint blue spots, first the 1-phosphohistidine and later the 3-phosphohistidine.

The peptides were hydrolysed in sealed tubes with 6 N HCl for 24 hours, and the amino acids separated by thin-layer chromatography on Fixion X 8 ion exchange resin coated plates, run and coloured as described by Sajgó—Dévényi (1972).

Finally the ^{32}P-labelled myosin was prepared only from long. dorsii of rabbit muscles. 0.75 millicurie inorganic ^{32}P per kg was injected into the peritoneum of a rabbit of 2400 g body weight. The rabbit was sacrificed 71 hours after injection, then myosin prepared, and radioactivity was determined in the fractions of chromatographied myosin at the National Frederic Joliot-Curie Ray-Biological Institute.

Results

Starting from 8.72 g dry myofibril powder, after hydrolysing with NaOH we removed the undigestible residue by centrifuge, and having carried out the necessary cleaning obtained 1.82 g (21.3 per cent) collagen. The phosphorus content of the lipid-free myofibril is an average of 21.3 microgramatom P per g dry protein, while without lipid solvents the phosphorus content can only be determined approximately, and this value will be between 30 and 60 microgramatom P per g protein. In both cases the value of dry myofibril naturally includes the collagen content.

The chromatographied and lipid-free myosin gives an average value of 10.3 g atoms P per 5×10^5 g dry protein, while the phosphorus value is 12.86 g atoms without chromatography and 20—24 g atoms without lipid solvent treatment; finally, without chromatographic purification and lipid removal varying (30—40 g atoms P) values are obtained.

After the removal of the collagen considerable quantities of large molecular weight peptides are still contained in the hydrolysate of the myofibril, therefore the substances disturbing the chromatographic separation were removed by silicotungstate reagent (Martin—Doty 1949). The subsequent operations were similar to those described in the methodical part: the hydrolysate was filtered on a Dowex 50×8 column, and after diluted divided into two equal parts, then chromatographied on a Dowex II×8 column. Half of the hydrolysate was chromatographied with the linear gradient method (Fig. 1) while the other half by stepwise elution, each with a 20 ml solution series of increasing concentration (Fig. 2). The amounts of amino acids and peptides were followed in 0.2 ml eluate by ninhydrin reaction according to MOORE—STEIN (1948), and read on a standard curve prepared with leucin. Both in the linear and the concentration gradient eluate fractions three peaks of amino acid and phosphorus content are shown. On both columns — in spite of the silicotungstate treatment — a considerable amount of phosphorus can be found in unhydrolysed large molecular weight peptides regained only with the regeneration of the column.

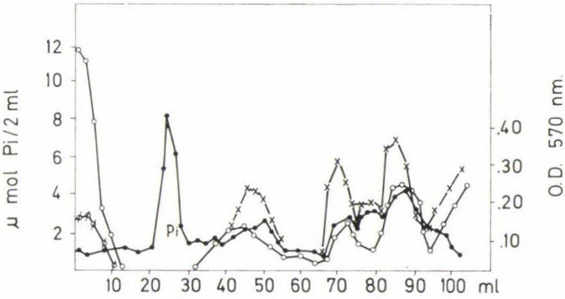


Fig. 1. Chromatography of myofibrillar hydrolysate applied on 1.2×12 cm Dowex 1×8 (Cl^-) column after having passed through Dowex 50. The hydrolysate elution was performed with 20 ml of 0.20—0.80 M $KHCO_3$ with a step by step method, as shown on the chromatogram. .—. Pi without hydrolysis; the further P after hydrolysis in 1.0 M HCl; o—o ninhydrin positive material at 570 nm; ∅—∅ arginine positive fractions x—x O. D. at 235 mm

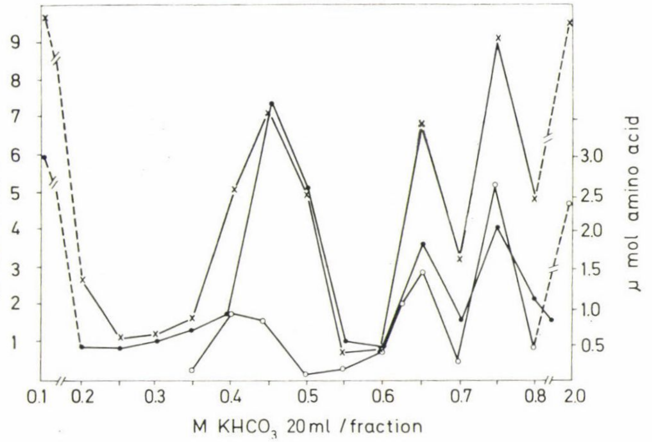


Fig. 2. Chromatography of alkaline hydrolysate of myofibril. Elution was performed step by step of 20 ml of 0.20—0.80 M $KHCO_3$. o—o ninhydrin positive fractions; other signs are seen in the figure

The chromatogram of the hydrolysate of 10.16 g dried myosin powder is shown in Fig. 3. The hydrolysis of myosin with 3 M KOH is not complete, as considerable further quantities of dissolved material can be precipitated by the perchloric acid and silicotungstate tests, and further phosphorus-containing peptides eluated from the columns with 2 M $KHCO_3$ or 0.1 M NaOH. Of the specific amino acid reactions the Sakaguchi reaction carried out according to ROSENBERG *et al.* (1956) pointed out the presence of arginine in the initial phase of the curve. By chromatographying the hydrolysate with

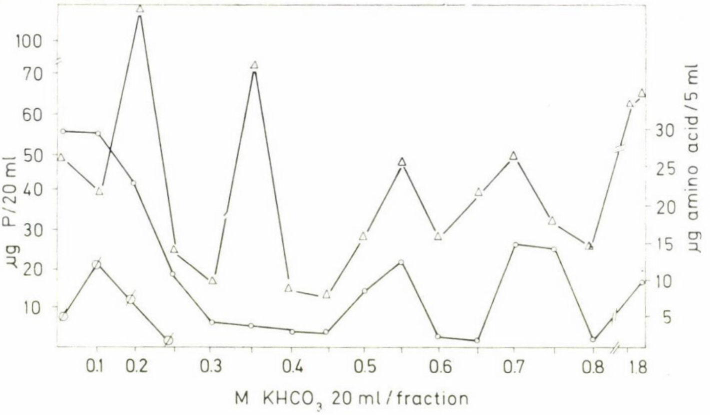


Fig. 3. Chromatography of 3 N KOH myosin hydrolysate on Dowex I × 8 column with $KHCO_3$ gradient elution. o—o amino acid, △ — △ free and bound phosphate, ∅ — ∅ arginine positive fractions

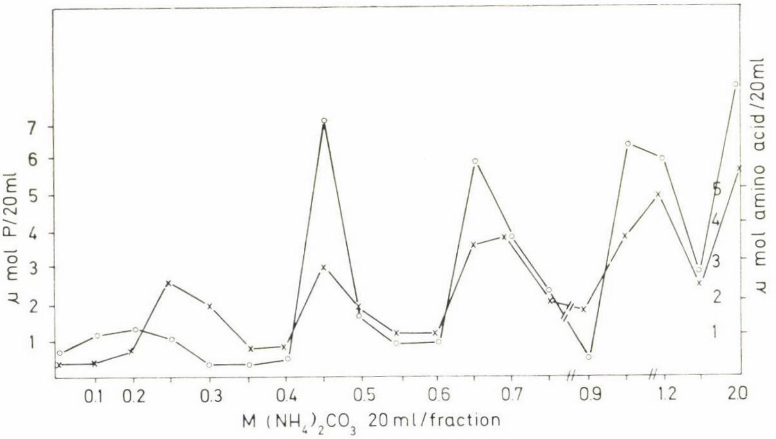


Fig. 4. Chromatography of 3 N KOH myosin hydrolysate on Dowex I × 8 column, with $(NH_4)_2CO_3$ gradient elution. 0.0 ninhydrin positive reaction in fractions, × — × phosphate content in fractions

a gradient series of ammonium carbonate solution a chromatogram similar to those in Figs 2 and 3 is obtained. (Fig. 4.) A great disadvantage of this method compared to the former ones is that the amino acid content of the fractions can only be determined after the removal of the salt. The presence of arginine in the initial phase of the chromatogram could be detected again.

In subsequent experiments we intended to determine the total phosphohistidine content of myosin. To attain this aim we omitted the silicotungstate precipitation of proteins (peptides) and the use of phosphate buffer in the extracting solution in order to avoid the superfluous introduction of phosphate in the myosin preparation. Myosin was extracted with 2 vol. of 0.6 M KCl solution alone. (Myosin extraction is carried out in the same way in Perry's laboratory when protein phosphorylation is the aim of the experiment.) Myosin was precipitated by dilution and lyophilized without further purification; the 19.1 g myosin powder thus obtained was used subsequently as crude myosin. Having performed the lipid removal we obtained 1.23 g slightly yellow oil and 12.12 g myosin powder which contained 140 mg (= 4.68 millimoles) phosphorus corresponding to 24.76 g atoms P per 5×10^5 g protein value, while the crude lyophilized preparation contained 44.5 g atom P per 5×10^5 g protein. The preparation was hydrolysed without further purification. After filtering the hydrolysate on a Dowex 50 column 97 per cent of the phosphorus content was recovered in the eluate. The eluate — after properly diluted — was applied on a Dowex 1 column and with gradually increasing concentrations of ammonium acetate (pH maintained above 8.6) eluated to

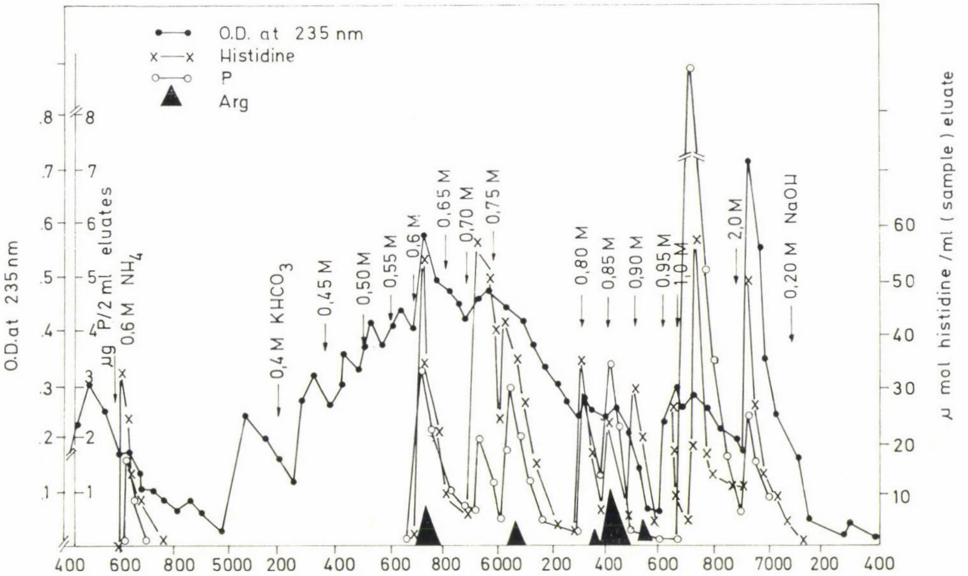


Fig. 5. Chromatography of hydrolysate of myosin on 2.2×30 cm Dowex I×8 column. The hydrolysate was applied on the column after having passed through Dowex 50 column, and measured on the basis of optical density at 235 nm. The elution was performed with the graduated concentration of ammonium acetate and $KHCO_3$ as shown on the chromatogram. The fraction was collected by the histidine and phosphorus content of the effluent . . - . O.D. at 235 nm

a concentration of 0.5 M. Subsequently we changed over to $KHCO_3$ elution; this phase is shown in Fig. 5. From 4400 to 7400 ml effluent 100 ml effluent fractions were collected. The histidine and phosphate contents were collected. The histidine and phosphate contents were determined in aliquot volumes in each fraction, within the range of 0.6—2.0 M $KHCO_3$. When summed up and detailed calculations omitted 2.931 g atom, with fractions obtained by 0.6 M ammonium acetate included 3.156 g atom phosphorus and 24.04 M histidine were found per 5×10^5 g protein value. The 0.9 and 0.95 M fractions did not contain phosphorus, only histidine.

It has become clear from the experiment that only a part of the phosphate remains in a stable form of covalent linkage after the alkalic hydrolysis, which can be chromatographied within the range of alkaline, on the other hand the measurement data have revealed that even a much lower quantity of initial material is sufficient to determine the histidine phosphorus content in the individual fractions, and this makes the quantitative determination from a smaller amount of myosin chromatographied on DEAE-cellulose column possible. Next time we started the experiment with 3.19 g of chromatographied, dialyzed, lyophilized and lipid-freed myosin. This amount contained 67.5 micromoles phosphate which is equivalent to 10.8 g atoms of phosphorus per 5×10^5 g myosin. When run over a Dowex column nearly 65.35 micromoles phosphate was recovered in the eluate. After adequate dilution the eluate was applied on a Dowex I column which fixed the phosphorus-containing peptides. Utilizing our previous experiences we washed the column with 2 millimoles NaOH until the extinction value of the effluent fell to 0.1 at 235

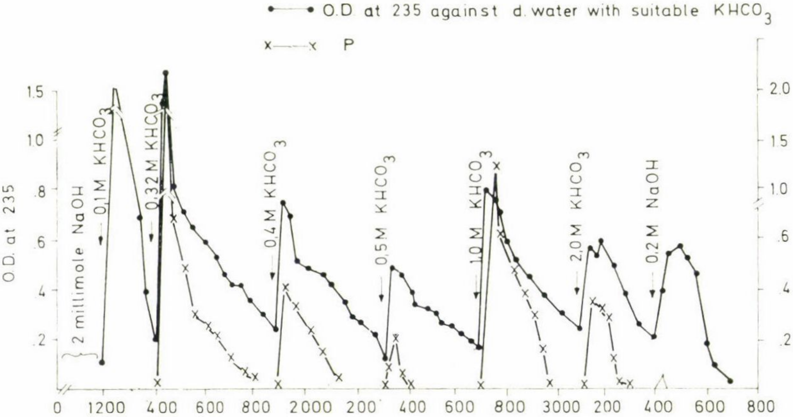


Fig. 6. Chromatography of purified myosin hydrolysate on 2.2×14 cm Dowex 1×8 (Cl^-) column. The hydrolysate was applied on the column after having passed through a Dowex 50 column, as shown in Table 1. Elution was performed step by step. Fractions containing histidine and phosphorus were collected. . - . O. D. at 235 nm, ×—× free and bound P

nm, and from then on performed a fractional elution as shown by Fig. 6 and summed up in Table 1.

The chromatogram of Fig. 6 contains 133 micromoles histidine in the region of phosphorus-containing fractions, which corresponds to 20.5 M histidine per 5×10^5 g protein. It should be emphasized that this value does not mean the total histidine content of the hydrolysate, and even the histidine content of the myosin is but partly represented by it. As seen from the data of Table 1, the amount of covalent linkage phosphorus is 23.56 micromoles — or with the 0.5 fraction included 25.66 micromoles — per 3.19 g protein, which corresponds to 3.72 g atoms and 4.02 g atoms per 5×10^5 g protein, respectively. On the basis of the data 4 fractions of the 20 M histidine are likely to have contained covalent linkage phosphate in the 0.6—2.0 M area of the chromatogram. Phosphates found in the other fractions quickly reacted as inorganic phosphates to the acidic ammonium molybdate reagent without hydrolysis under the usual conditions. This fraction can otherwise be eluated from the Dowex 1 column in a separate experiment at a 0.15 M $KHCO_3$ concentration. We can see from the data of the table that peptides containing phosphate in a combined form make a relatively small proportion — 4.69 per cent when calculated on the basis of optical density — of the hydrolysate. Closer examination reveals that 80 per cent of the phosphopeptides

Table 1

Phosphorus content in the total hydrolysates during separation

	Total ext./fractions				Max. absorbance at × nm	P content/fr. micromole
	at 235 nm		at 280 nm			
Hydrolysate (3.19 g)	9785	(100%)	4400	(100%)	216 nm	67.5
Dowex 50 removal	1335	14.6				
Appl. for Dowex 1 column	8365	85.5	497			65.35
Effluent fr.						
unbound fr.	2140	22.0				
2 millimoles NaOH	4845	49.5				
0.1 M $KHCO_3$	375	3.86	95	2.16	227	0.25
0.32 M $KHCO_3$	553	6.46			206	35.57
0.40 M $KHCO_3$	140	1.64			220	14.06
0.50 M $KHCO_3$	111	1.3			219	1.95
1.00 M $KHCO_3$	116.65	1.35			221—225	20.2
2.00 M $KHCO_3$	158.8	1.52			224—227	3.3
0.2 M NaOH	43.4	0.52				0.11

is found in the 1 M $KHCO_3$ fraction which hardly amounts to 1.5 per cent of the hydrolysate.

In our next experiment we reduced the 1 M $KHCO_3$ fraction to further fractions by gradient chromatography. This fraction found small on the basis of optical density but containing 20 micromoles of phosphate was applied on a small volume Dowex I column of 1×3.3 cm and separated into fractions according to concentrations indicated on the chromatogram. Only fractions containing phosphorus were collected (Fig. 7).

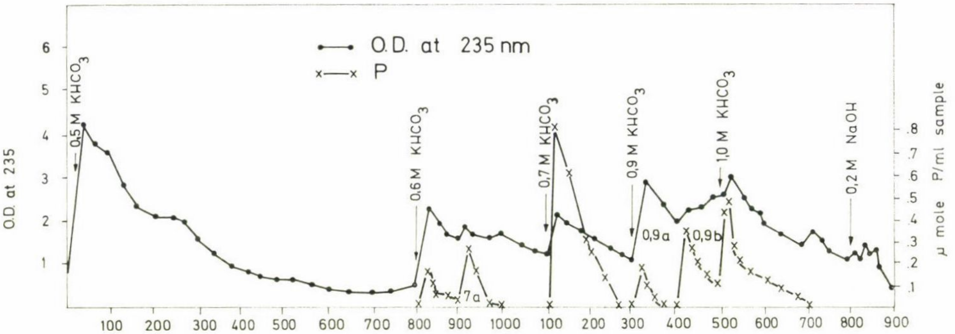


Fig. 7. Rechromatography of 1 M $KHCO_3$ fraction of purified myosin on 1×3.5 cm Dowex 1×8 (Cl^-) column. Applied on column = 159.1 at 235 nm, selected from fractions of Fig. 6. Gradient elution technique was used as shown on the chromatogram. The chromatogram shows the phosphorus content of the fraction collected and kept for further investigations

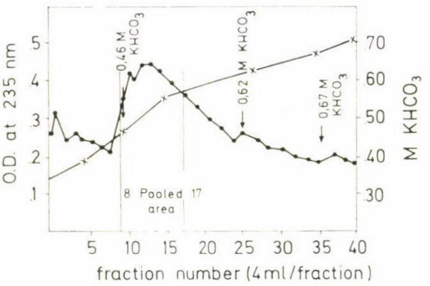


Fig. 8. Linear gradient chromatography of the 0.5 M $KHCO_3$ fraction of purified myosin. The fraction was applied on 1×2.6 cm Dowex 1×8 column. This fraction was obtained by the 0.5 M $KHCO_3$ solution as shown on Fig. 6. The marked area contains phosphorus collected for further investigations . - . O.D. at 235 nm

Further on fractions 0.5 M $KHCO_3$ (Fig. 8), 2 M $KHCO_3$ (Fig. 9) and 0.32 M $KHCO_3$ (Fig. 10) were submitted to chromatographic purification by the linear gradient method. In the figures we indicated the phosphorus contents of the fractions and the concentration of $KHCO_3$ which was determined titrimetrically in aliquot volumes. The fractions of the latter four figures (Figs 7—10) were found to be heterogeneous by the paper chromatographic control (we note here that the 0.45—0.75 M peptide fractions* collected by gradient elution of myofibril and myosin shown in the previous figures were also heterogeneous). In order to be able to separate the total quantities of the individual fractions by chromatography we removed the

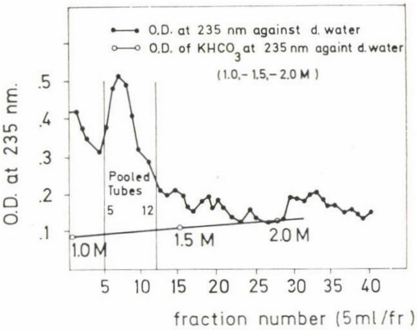


Fig. 9. Linear gradient chromatography of 2.0 M $KHCO_3$ fraction from Fig. 6. on 1 × 2.6 cm Dowex 1 × 8 column, obtained from purified myosin. The marked area contains phosphorus collected for further separation by paper chromatography. $KHCO_3$ concentration was measured by titrimetry and checked and shown at 235 nm on the basis of potical density; it is indicated by . —— .

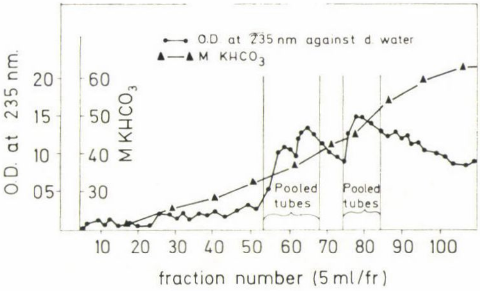


Fig. 10. Linear gradient chromatography of 0.32 M $KHCO_3$ fraction (Fig. 6) on 1 × 2.5 cm Dowex 1 × 8 column. Both areas contain phosphorus collected for paper chromatography. The concentration of $KHCO_3$ — marked △——△ on the chromatogram — was traced titrimetrically

$KHCO_3$ by adding dry Dowex 50 ion exchanging resin to the fractions — cooled in icy bath and stirred with a magnetic stirrer, then controlled by direct reading pH meter — to pH 6.5, then having removed the resin restored the pH of the supernatant to 9 with KOH and lyophilized the samples. The lyophilized samples were dissolved in a minimum 0.1 M NH_4OH solution and separated on Whatman No. 3 paper as described in the methodical section.

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* Besides histidine phosphates they contained peptides too.

The presentation of the chromatogram series would be meaningless. Fig. 11 is sufficient to elucidate the principle and result of separation. During 19—20 hours of running the solvent front reaches 27—29 cm and the peptides separate in sharp lines. The stripes were cut out and suspended — as usual with descending chromatograms —, the peptides were eluated with an approximately 15 per cent NH_3 solution and collected in a cca. 0.5 ml effluent solution. From each of the separated peptides a sample was taken and its phosphorus and histidine content determined. Starting from myosin obtained by chromatographic separation we had a total of 49 peptide stripes of which, however, only 26 contained histidine and phosphate. The fractions up to the 0.9 M fraction contained more or less inorganic phosphate. As found out later, the appearance of the inorganic phosphate is in connection with the presence of histidine phosphate. Phosphopeptides proved to be much more stable than phosphohistidine. Histidine phosphate was contained in fractions 0.5, 0.6, 0.7 and 0.75 M; the fraction 0.5 M contained 3-P-histidine with 0.55 Rf value, while the other fractions 1-P-histidine with 0.31—0.36 Rf value (Fig. 12).* When run with synthetic histidine phosphates the isolated fractions run with the same Rf value, and having been hydrolysed contain a single amino acid, histidine. When sprayed with ninhydrin reagent the histidine phosphates show a well distinguisged blue staining. In adequate quantities yellowish spots of inorganic and pyrophosphates can also be seen. We observed the in-

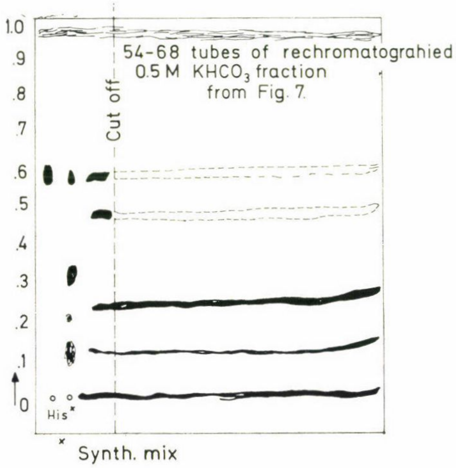

Fig. 11. Paper chromatography of peptides of 54—68 tubes of rechromatographied 0.5 M $KHCO_3$ fraction from Fig. 8. The two upper stripes were detected by ninhydrin reaction

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* The Whatman No. 3 paper is more suitable for the separation of P-histidine and its peptides; see Fig.

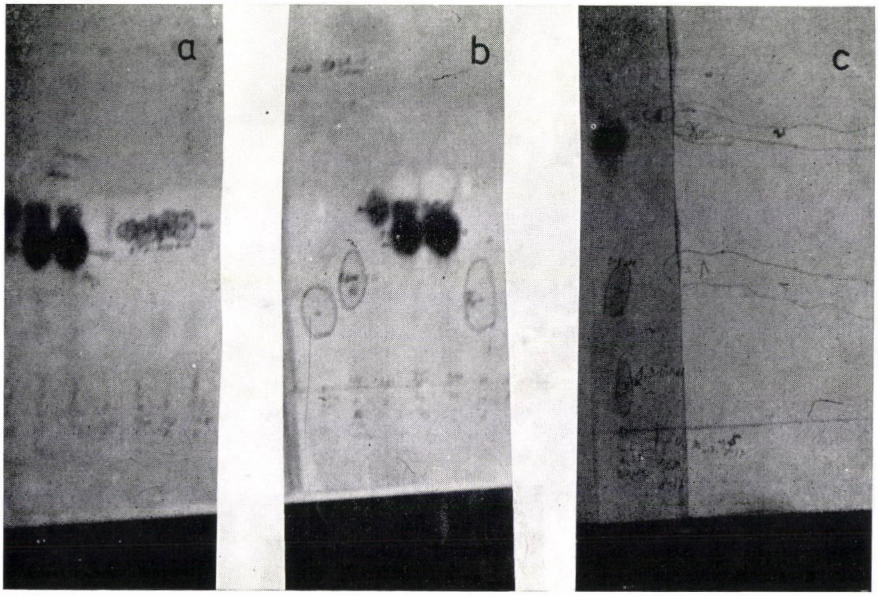

Fig. 12. Paper chromatography of isolated and synthetic phosphohistidine sand peptides. *a)* Histidine, mixed histidine and arginine; two spots of synthetic 3-phosphohistidine, and two spots of the mixture of synthetic and isolated 3-phosphohistidine, run on Whatman No 1 paper. *b)* Inorganic phosphorus, 1-phosphohistidine, histidine, mixture of histidine and arginine, 1,3-diphosphohistidine run on Whatman No 1 paper. *c)* Spots of arginine, 1-phosphohistidine, 1,3-diphosphohistidine, and lines of two isolated phosphopeptides run on Whatman No. 3 paper

organic and pyrophosphate elution of synthetic phosphohistidine from Dowex I column in separate experiments and registered their paper chromatographic Rf values (Table 2).

We carried out amino acid analyses of some peptides. Many of them require further purification. One of the peptides — of relatively short 0.32 Rf — was found to be suitable for further comparison; its amino acid composition is shown by the following table.

Phosphohistidine peptide from myosin

	μM per sample
Asp	.115
Ser	.039
Glu	.198
Gly	.041
Ala	.035
Val	.054
Ile	.035
Leu	.064
His	.028
Lys	.035

Summarizing the results of experiments we found that the phosphorus-containing polypeptide chain showed a rather regular hydrolysis, and the peptides differed from each other in length only. We noticed that the shorter peptides did not absorb the UV light between 260 and 300 nm, that is, they did not contain aromatic amino acids. The longer ones, on the other hand, contained acidic amino acids cumulatively, and the amount of lysin in them was usually twice that of the histidine. By protracting the hydrolysis, the number and length of the basic peptides can be reduced which, however, do not lead to a proportionate increase in the amount of histidine phosphate. RATHLEV—ROSENBERG (1956) pointed out that the phosphohistidines were apparently hydrolysed — even if slowly — in an alkaline medium to. We decided therefore that instead of studying the favourable conditions of hydrolysis for the phosphopeptide chains we would try to separate homogeneous phosphopeptides by means of enzymes.

Table 2

Rf values of phosphohistidines and phosphates and their elution concentrations obtained with $KHCO_3$ from Dowex 1 column

Material	Rf	$KHCO_3$ conc.
Histidine	0.58—0.61	to 0.26 M
3-P-his	0.54—0.57	0.64—0.70
1-P-his	0.31—0.36	0.47—0.55
1,3-di-P-His	0.20—0.23	—
Pi	0.09—0.13	0.13—0.15
PPi	0.00—0.05	0.57
ADP	—	0.38—0.40
P-Arg (synt.)	0.70—0.73	0.05—0.10
P-Lys (synt.)	0.74—0.80	0.35—0.40

In order to avoid the possible heterogeneity of myosin we prepared it from a single muscle *(long. dorsii)* and isolated the heavy chain from the largest of the chromatographic fractions. The phosphopeptide was obtained by trypsin digestion as described in the methodical section; total digestion was carried out. The digest of 830 mg heavy chain was applied on a column made of 5 g DEAE-cellulose (1.3 × 18 cm) and equilibrated with 1 millimole Tris-HCl buffer (pH 8.6). The heavy chain contained 378.5 μg phosphorus which corresponds to 12.2 g atom P per 4.1×10^5 g protein (Fig. 13). The details of the chromatographic separation and the distribution of the fractions are shown in Table 3.

Table 3

Distribution and percentage recovery of chromatographic fractions of tryptic digest from heavy chain

Fraction	O. D. at 235 nm	%
Applied for column	372.6	100
Unbound fraction	129.0	34.4
Wash-buffer of column		
fr. I. (100 ml)	45.6	12.52
fr. II. (100 ml)	15.0	4.0
Chromatographic fractions:		
linear gradient with NaCl		
peak a. 1—14 tubes	34.0	9.2
peak b. 15—24 tubes	38.7	10.3
Conc. gradient with NaCl		
0.3 M 25—60 tubes	45.6	13.0
1.0 M 61—64 tubes	9.30	2.5
1.0 M 65—70 tubes	19.30	5.25
2.0 M 71—75 tubes	9.23	2.47
0.2 M NaOH	6.0	1.6
	351.73	94.5

On the chromatogram four fractions containing phosphorus were found. The paper chromatographic examination of fraction 1 obtained by linear gradient showed stripes containing histidine, histidine phosphate, inorganic phosphate and peptide. After dissolution and HCl hydrolysis the broad stripe of the histidine phosphate contained a single amino acid, and it was only histidine. Fractions 2 and 3 contained an insignificant quantity of phosphorus-

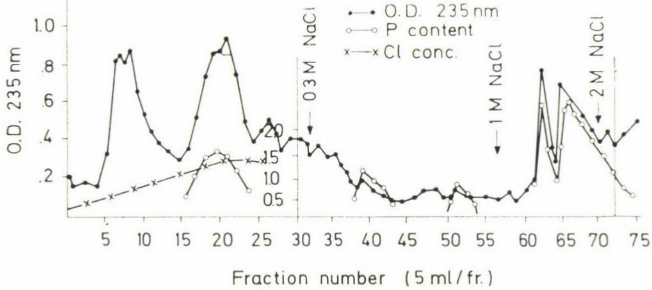

Fig. 13. Chromatography of tryptic digest of myosin heavy chain on DEAE-cellulose column E_{52}, 1.8×25 cm, equilibrated in 10 mmol Tris-HCl (pH 8.0). Elution was performed with 0—0.15 M NaCl linear and with graduated concentration as shown on the chromatogram

containing material which was not analysed. Fraction 4 — eluated with 1 M NaCl — was the most significant. The fraction was treated as a uniform material, collected in 60—70 tubes, lyophilized and for the purpose of desalting gel-filtrated on a Sephadex G 25 column (Fig. 14a). We found that the peptide really eluated in two peaks but one of them contained a considerable amount

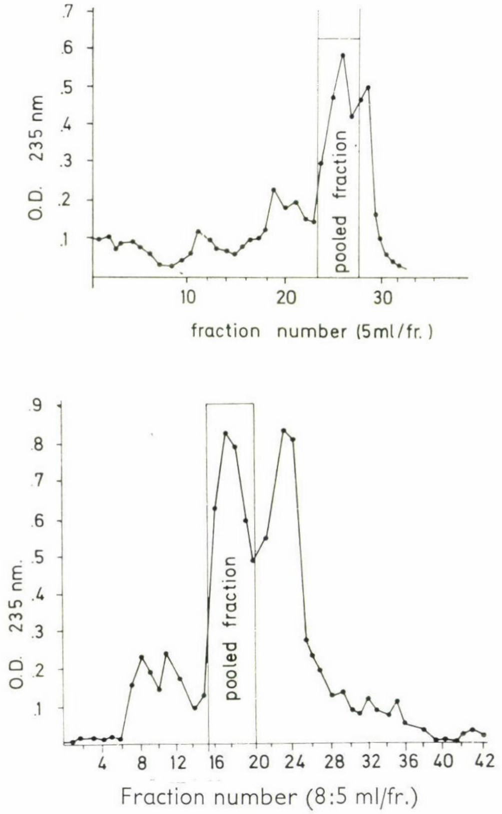

Fig. 14. Gelfiltration of tryptic phosphopeptide from 62—70 tubes of Fig. 13. *a)* The gelfiltration of phosphopeptide was performed on Sephadex G-25 (1.8 × 66 cm = 170 cm^3) column. *b)* Repeated gelfiltration of phosphopeptide on the same Sephadex G-25 column

of salt. Therefore from the gel-filtrated fractions only the five tubes indicated on the chromatogram were collected, lyophilized, then dissolved in a minimum volume (4 ml) on the same Sephadex column repeatedly gel-filtrated and 5 ml fractions collected (Fig. 14b). Although in this way a considerable part of the lower molecular weight fraction was lost, still the phosphorus-containing peptide repeatedly eluated with two peaks, and a part of it together with salt.

The phosphopeptide fractions shown in the *b)* figure were collected and separated on Whatman No. 3 paper. On the chromatogram a narrow fluorescent and two broad, hardly fluorescing stripes can be distinguished. The narrow stripe is found at a distance of 5.5 cm, the second at 10 cm while the third one at a medium distance of 18 cm when the front is 29 cm. After the elution of the peptides the examination revealed phosphorus content in all three stripes, the largest quantity in the second one, while after HCl hydrolysis, according to its specific reaction the first stripe consisted exclusively of histidine, the second one of histidine phosphate while the third fluorescent stripe proved to be a peptide of phosphorus content (Fig. 15).

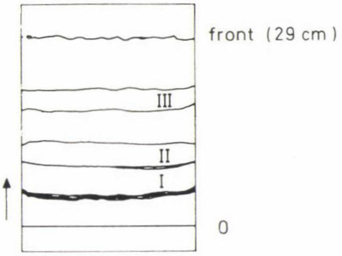

Fig. 15. Paper chromatographic separation of gelfiltrated phosphopeptide on Whatman No 3 paper. Stripes detected by UV light: I = strong fluorescence, II and III = weak fluorescence stripes

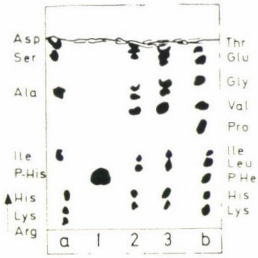

Fig. 16. Thin-layer chromatography of hydrolysate of tryptic peptide I. stripe on Fixion X 8 ion exchange resin coated plates. The samples were hydrolysed for 24 hours in 6 N HCl in sealed tubes. 2, 3 different quantities of sample, a and b amino acids as controls. 1 . . synthetic 3-phosphohistidine

After hydrolysis the chromatogram shows Lys, His, Leu, Ile, Val, Ala, Gly, and probably Glu, Ser (Fig. 16).

By introducing 0.75 millicurie ^{32}Pi per kg body weight myosin was prepared from a single muscle of a rabbit of 2400 g weight. 71 hours after injection the rabbit was sacrificed and the labelled myosin was prepared from the long. dorsii (110 g). 0.58 per cent (= 10.57 μc P) of the total activity introduced with the injection (1800 microcurie/2400 g) was found in 110 g muscle at the beginning of the preparation, while in the blood 1/13/total body vol. (13 μc) = 0.73 per cent. In the course of the preparation the myosin showed a behaviour different from the usual inasmuch as more protein than actomyosin precipitated and more protein was removed by the ultracentrifugal purification. Part of the ultracentrifuged myosin was separated by column chromatography. On the chromatogram the fractions separated unusually well (Fig. 17) and we were surprised to find that the incorporation of the phosphate was the highest in two small fractions, and the chromatographically pure myosin (fraction III) — while hardly showing double the radiation of the background (9 cpm/mg) — contained 13 gramme-atom phosphorus per 5×10^5 g protein. The figure shows the labelled fractions and numerically the extent of incorporation. Although fraction V shows a considerable phosphate incorporation, it must be excluded from the evaluation of the experiments because according to its ATPase activity its myosin content is only 30 per cent of that of fraction III but contains considerable quantities of lipid and nucleic acid. In the course of the SDS gelelectroforesis the most significantly labelled fraction showed a stripe — supposedly the ML_2 fraction — at the height of the light chain (small) fractions. The previously eluated small fractions possessed significant acetylcholin esterase activity.

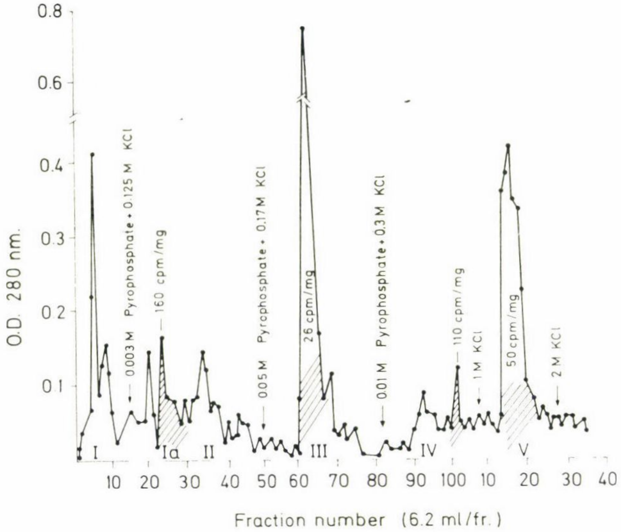

Fig. 17. Chromatography of ^{32}P-labelled myosin on DEAE cellulose column. The elution was performed as reported by FAZEKAS *et al.* (1971). Acta Agronomica Acad. Sci. Hung., *20*, 271—283

The phosphorus content of myosin greatly depends on the conditions of preparation and the lipid content of the crude myosin. From phosphate buffer containing fractions obtained by means of chromatography the phosphate cannot be removed by exhaustive dialysis, on the contrary, the latter leads to a state of equilibrium, therefore we thought it proper to carry out the following examinations to find out the covalent-linkage phosphorus content of myosin.

Before any further examination, the myosin prepared exclusively from *long. dorsii* was centrifuged at 105 000 g for two hours, and the thus purified myosin was divided into two equal parts.

1. *a)* One of the parts was cooled, adjusted to a final concentration of 5 per cent with ice-cooled 20 per cent TCA*, then the myosin was precipitated and removed by centrifuge. The precipitate was dispersed in 5 per cent TCA and centrifuged on further two occasions, finally dried several times in ether and air, then its protein and phosphorus contents were determined. The results are given as calculated for 5×10^5 g protein. The preparation contained 29.4 g atoms phosphorus. As a matter of interest we mention here that this amount of protein could be obtained from 878 500 g air-dried myosin powder (1b). The ether-extracted myosin powder was suspended in 1 N NaOH and hydrolysed in a 100 °C water bath for 20 minutes. It was then cooled to room temperature, its pH adjusted to show an acidic reaction, and the protein was precipitated with 0.3 vol. ice-cooled 70 per cent perchloric acid and centrifuged. The precipitate was repeatedly washed with 1 per cent ice-cooled perchloric acid then with ether, dried, and the phosphorus content of the preparation determined. It contained 2.8 g atoms phosphorus per 5×10^5 g protein.

2. *a)* The other part of the myosin was freed from nucleotid, nucleic acid, salt, lipid and phospholipid by the method of KLEINSMITH *et al.* (1966) as detailed below.

1. The protein was precipitated with 20 per cent TCA to a 5 per cent concentration, and centrifuged.
2. The precipitate was dispersed in 5 per cent TCA, and extracted several times. After each extraction the precipitate was centrifuged and collected.
3. To the precipitate 1 N NaOH was added until it was dissolved, then diluted ten times.
4. Adding 20 per cent TCA to a final concentration of 5 per cent the myosin was precipitated and centrifuged.
5. The precipitate was dispersed in 10 volumes of 10 per cent TCA and hydrolysed in a water bath of 90 °C temperature for 15 minutes, then cooled and centrifuged.

6—8. The precipitate was extracted with lipid solvents, and after each extraction collected by centrifuging. It was extracted with 20 volumes of aceton, 20 volumes of ethylether, 20 volumes of chloroform–metha-

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* Tri-chloro-acetic acid.

nol (2 : 1, v(v) mixture, and finally with ether in the above succession, and dried to steady weight. The phosphorus content when determined was found to be 138 g P (= 4.6 g atom P) per 5×10^5 g protein.

2. *b)* The myosin powder was further treated. It was dissolved in 1 N NaOH and hydrolysed in a water bath of 100 °C temperature for 20 minutes, then cooled and adjusted with 4 M HCl to pH 6.0.

The protein was precipitated in an ice-bath with 0.3 volumes of ice-cooled 70 per cent perchloric acid, then centrifuged. The precipitate was suspended in ether, then treated with ether and dried. It contained 82 g phosphorus (= 2.7 g atoms) per 5×10^5 g protein.

When comparing the results we find that the phosphorus content of protein obtained after the alkaline hydrolysis was nearly the same in the two experiments (2.8 g atom). The latter experiment shows, in addition, that the alkaline hydrolysis removes about (4.6 – 2.7 =) 1.9 g atoms ester-bound phosphate from the lipid-free myosin.

3. *a)* The next control experiment was carried out with myosin prepared from 600 g mixed muscle. By stirring the ultracentrifuged 350 ml myosin with 150 ml methanol the protein was denaturated, then by continued stirring with 300 ml chloroform added the lipids were extracted and centrifuged. The salt-containing watery phase was poured out and the organic phase containing the lipids was collected. The precipitate was repeatedly extracted with 20 volumes of a mixture of chloroform and methanol (2 : 1, v/v) and centrifuged. The protein residue was extracted several times with ether and dried; 20 g dried myosin powder was obtained.

The lipid-containing organic phase was unified, evaporated, and after repeated extraction, distillation and drying 1.45 g lipid was obtained which corresponded to 36 250 g lipid per 5×10^5 g protein.

When analysing the phosphorus content we found only 4.8 per cent phospholipid* (1812 g = 2.11 M^+) and 95 per cent other lipids.

3. *b)* The lipid-free myosin powder was further analysed, dissolved in 1 N NaOH, brought up to 50 ml, hydrolysed at 37 °C for 18 hours, cooled, adjusted with HCl to show a mild acidic reaction, and precipitated with perchloric acid to a final concentration of 5 per cent, then after 20 minutes of centrifuging at 5000 g the precipitate was collected. The sticky precipitate was treated with ether, the dry residue weighed (5.9 g) and its phosphorus content determined (701 μg). The phosphorus content corresponds to 59.24 g Pi (= 1.94 g atom) value per 5×10^5 g protein.

The ratio of protein and phosphorus was determined in the non-hydrolysed residue and the hydrolysate. In 172 500 g non-hydrolysed protein res-

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* We related the molecular weight of phospholipid to lecitin and took it to have an average molecular weight of 860.

idue 59.84 g P (1.94 g atom), in 327 500 g protein hydrolysate 254.5 g P (8.5 g atom), altogether in 5×10^5 g protein 305.34 g (10.44* g atom) phosphorus was found. The 2.11 M phospholipid phosphate can be included in the phosphate content of myosin. The adsorbed phosphate was poured out with the watery phase obtained with the lipid extraction.

Discussion

It is a well known fact that the removal, identification and quantitative determination of the accompanying materials of the myofibrillar origin protein preparations represent one of the most difficult tasks and prevent us from detecting the properties of the pure protein, carry out its reliable analysis and observe refined changes in its structure. The purification of myosin by cyclic precipitation reduces the quantities of its accompanying materials only to a low extent, but series of this process involve great losses of material, while in the course of precipitation with TCA a large proportion of the secondary materials is aggregated together with the protein. These methods do not render the separation of the absorbed phosphate and the control of its quantity possible. That is why we were surprised to find that with the technique of KLEINSMITH *et al.* (1966) 42.3 per cent of the dry weight is removed from the myosin and 4.6 g atoms phosphate is left behind. The full completion of this experiment results in the removal of further 2 g atoms or so phosphorus from the myosin — in the case of 1 N NaOH treatment performed at 100 °C for 20 minutes.

The presence of large quantities of associate materials could earlier be concluded on from chromatographic purification performed on DEAE-cellulose too, insofar as in the three chromatographic myosin fractions obtained from mixed muscle only 45 per cent of the initial material was recovered (FAZEKAS *et al.* 1973). The chromatographic fractions contained 8—12 M phosphate each. In spite of the fact that before the alkaline hydrolysis many components are removed — lipid, phospholipid, nucleic acid and nucleotide — the quantity does not correspond to the weight of the removed materials only together with the dry weights of salt, buffer and of proteins soluble in the used solvents. Besides the above, other experiments also confirm that 10.8—11 g atom is the probable value of phosphate of covalent bond in myosin.

These data are in themselves sufficient to suggest that the ATP-ase activity cannot be the only function of myosin, and encourage us to continue dealing with the absolute phosphorus content of myosin instead of changes

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* 10.8 g atom phosphorus was found in the lyophilized and lipid-free myosin too before hydrolysis.

in its phosphorus content and trying to obtain fractions of high purity with a reliable phosphorus content, which can serve as a starting point in our further investigations. The inadequacy of the purification techniques and the pH dependence of the phosphorus content led us beyond the fine chromatographic methods to the application of rough purification techniques and, at the same time, to the existence then isolation of histidine phosphate.

We were confirmed that we were following the right track by the experimental results of MÜHLRAD *et al.* (1964) who — in spite of the similarly rough perchloric acid treatment used — observed considerable covalent bond phosphate formation in the myofibril, and held the myosin responsible for the incorporation of P^{32}. This theory is supported by our present results which confirm that in the lipid-free myosin 10—11 g atom covalent bond phosphate occurs of which 4 g atoms are phosphohistidine. Phosphohistidine and its peptides can be isolated from an alkaline hydrolysate. If from the hydrolysate the large peptides are removed by silicotungstate, on a Dowex I ion exchanging resin column, at a $KHCO_3$ concentration of 0.2—0.8 M a fraction containing inorganic phosphate and two or three fractions of amino acid phosphate are obtained (Figs 1, 2, 3 and 4). Fractions 2 and 3 consist almost exlusively of 1-phospho-, and 3-phosphohistidine, respectively. They are identified by paper chromatography and on the basis of their specific reactions.

These experiments provide no information on the quantitative conditions of the histidine phosphate. Therefore from the hydrolysates of the subsequent experiments we did not remove the large molecular weight fractions in order to be able to demonstrate the presence of all phosphate-containing peptides on a Dowex I column. By this method we pointed out that from crude myosin 3.15 g atoms, while from chromatographically pure myosin hydrolysate some 4 g atoms covalent bond phosphate per 5×10^5 g myosin could be obtained. Through a continued purification by column- and paper chromatography the histidine phosphates can be isolated and identified (Figs 11 and 12), but this method is too lengthy and laborious for using to obtain peptides of a purity and quantity suitable for sequence analysis. Although during the examination the phosphopeptides were found to split according to some rule, we could isolate — with a rather great loss — 26 phosphopeptides. In addition to the above described peptide of amino acid composition some shorter — but insufficient in number for further examination — phosphopeptides occur. Therefore we stopped purifying phosphopeptides obtained by alkaline hydrolysis and tried to isolate homogeneous phosphopeptide by means of enzymes.

The result of the isolation shows that the free phosphohistidine is also eluated from the DEAE-cellulose column. The phosphorus-containing fraction eluated in the low concentration range by the linear gradient method shows the same behaviour as the phosphohistidine of COLOMB *et al.* (1972) who separated it from heart cytosol under similar conditions. The main phosphorus-containing peptide fraction when studied by paper chromatography showed a narrow, highly fluorescing peptide stripe as well as one containing free phosphohistidine and histidine. Tryptic digestion — since it had not been stopped — only ceased after the gelfiltration, so the phosphohistidine production can be explained by this (Fig. 16). This suggests, at the same time, that somewhere in the myosin phosphohistidine is in the vicinity of lysine. It is known, otherwise, that the Lys-His-phosphate bond is highly trypsin resistant.

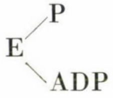
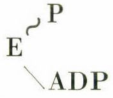
Since the isolation was carried out from the tryptic digest of a heavy chain the result shows that the phosphohistidine is originally in this chain. In the meanwhile isolation was carried out from the chromatographically pure myosin heavy chain of a single *(long. dorsii)* muscle too; as a result, after basic hydrolysis phosphohistidine was isolated. Phosphohistidine was thereby proved to originate from the heavy chain.

Finally, according to our incorporation experiments performed with radioactive phosphate (Fig. 17) the labelled phosphate is incorporated in two low molecular weight protein fractions and in fraction 5, the chromatographically pure myosin fraction hardly shows an activity larger than the background radiation. Of the low molecular weight proteins the fraction eluated at a low salt concentration was identified by polyacrylamide gel electrophoresis with fraction ML_2 owing to its position and molecular weight.

It remains to be decided how the results of our experiments fit into the so far known functional scheme of the myosin and the other myofibrillar proteins. To this the starting point is provided by the data of MOMMAERTS (1954), namely, the Ca-ATP-ase activity of the fresh myosin is pH dependent: the activity measured at 9.6 pH is about four times the maximum found at 6.3, though after seven days this maximum disappears and the activity becomes identical in a wide range (pH 6.3—9.7). In our opinion the extra Ca-ATP-ase activity can be explained by the decrease of the active form of histidine phosphate, since myosin is usually stored between pH 6.5 and 7.5.

The extra phosphate burst from myosin is generally known and accepted today. In short we refer therefore to the work of TAYLOR *et al.* (1970) only, according to which by Ca activation 1.8 M P per 5×10^5 g myosin of steady state is released from the myosin in 50—100 seconds, and two ATPs are bound to it. While KINOSHITA *et al.* (1969) observed the binding of 1 M ATP and the formation of 1 M active complex, they found 10—20 M phosphorus per 4×10^5 g protein to be released at 0—10 μM Mg ion concentration. This finding also confirms that the myosin is a phosphoprotein, and phosphohistidine of moderately stable bond is abundantly formed in it. In order to avoid misleading ourselves and others we have to give account of some experiences obtained during our work, namely that not only 4 M histidine phosphate remaining stable and demonstrable during the alkaline hydrolysis (with the exception of 2 serine phosphates) further covalent bond but decomposing phosphates too exist in the myosin up to 10—11 g atom phosphorus content. We consider this difference to be 1-phosphohistidine. Its decomposition during the protracted isolation and purification is caused by the steps unfavourable for it. It may seem to be an exaggeration that in the myosin 30—40 g atom phosphate of covalent bond can occasionally be measured by a direct method. This less steady phosphate is considered to be 1,3-diphosphohistidine. Its isolation requires still more alkaline conditions than in the case of 1-phosphohistidine, owing to the quarterner N. These two compounds are stable in a peptide chain owing to the peculiar environment, but in free form they are moderately stable. The former requires the maintenance of 9 (Hultquist 1968), the latter at least 11 pH during isolation (our own experiences concerning the synthetic 1,3-diphosphohistidine).

In the peptide chain of myosin we assume the existence of the following form of activated histidine which may explain the phosphorylation of the histidine and serine lateral chains, respectively. The latter form may explain the formation of p-nitrothiophenylglutamic acid isolated by Kinoshita *et al.* (1969), namely the reaction of glutamic acid with p-NTP which is thought to play an intermediary role in the myosin.

By the substitution of phosphohistidines in the myosin function scheme of Kinoshita *et al.* (1969), and others (Tokiva—Tonomura 1965, Bagshaw—Trentham 1974), the formation of the hardly active  $\mathrm{E}<^{\mathrm{P}}_{\mathrm{ADP}}$ and reactive  $\mathrm{E}<^{\sim\mathrm{P}}_{\mathrm{ADP}}$ forms of the myosin substrate complex (ES), the release and binding, respectively, of H^+ ions in the hydrolysis of ATP are easy to understand.

In our opinion instead of the simple A_1 and A_2 alkaline light chains (Dreizen *et al.* 1970, Weeds—Lowey 1971) their phosphorylated and non-phosphorylated forms take part* in the development of the ATP-ase activity, and accordingly ML_2** in the regulation. We agree with Tonomura *et al.* (1972) that in this process "the substrate moves about among the peptide chains during the whole mechanism".

We think that phosphorus accumulation is a more important function of the myosin, and the K values up to the Pi and the ADP dissociation, respectively, represent the process of phosphate filling rather than the ATP-ase activity. This theory and the presence of histidine phosphate seem to be con-

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* At present we have evidence in that direction which will be published next.

** Alternative term: DTNB chain.

firmed by the investigation results of MANNHERZ *et al.* (1974) who succeeded in carrying out the synthesis of ATP in Subfragment 1 by properly choosing the substrate concentrations.

TONOMURA *et al.* (1972) separated the two ways of the reaction mechanism of myosin ATP-ase according to the substrate concentrations. The reaction mechanism which consists of more than one step is realized at a substrate concentration below 1 μM. We are also of the opinion that BAGSAW—TRENTHAM's (1974) extended mechanism works at the low substrate concentration and it is in this way that the heavy chain of myosin is filled up with reactive phosphate. This may all the more to be case because the myofibril attains an ATP concentration higher than that only under the influence of some stimulus (release of Ca^{2+}, troponine regulation), and it is then that the enzymatic way depending on the higher concentration may come into effect. This opens, in fact, a new way of reaction with the participation of actin, since the "actin accelerates the dissociation of the end product" (LYMN—TAYLOR 1971). We can see here the biological meaning of the two types of mechanism, namely that in spite of a low substrate concentration the myofibril can be filled up with reactive phosphate, but the action joining in the process of contraction occurring under the influence of the Ca ion when work is performed changes the value of K_5 whereby the dissociation of the inorganic phosphate is accelerated. This changed K value and the further steps evoked by the actin are not properly assessed yet.

The idea of BÁRÁNY—BÁRÁNY (1959) of an active centre and the functioning of the ATP-binding peptide isolated by BÁRÁNY—MERIFIELD (1972) fit into this theory, but its origin should be decided since it was isolated from heavy chain obtained by trypsin digestion, and the authors did not examine whether or not it contained heavy chains.

In the above theory there are a great many agreeing data, but even more can be fitted in. For example — to mention only the data of PANTELEYEVA *et al.* (1974) from the most recent investigations — the participation of water containing H_2O^{18} in the ATP hydrolysis could not be demonstrated, all the more so was the possibility of the labelled O^{18} appearing in any O of the inorganic phosphate proved. This also confirms the wandering of the substrate in the multistep process. The experiments of MÜHLRAD—FÁBIÁN (1968) in which the Mg^{2+}-ATP-ase activity was increased twentyfold with trinitrobenzol sulphonic acid (TBS) as a consequence of the 4 lysine of myosin having been blocked can also be fitted in. This proves the vicinity of Lys-His-P. Furthermore, not only one but more His-P intermediary peptides should be observed, but they remain to be proved. There are, however, incompatible data too. Why is it in the light chain that P^{32} appears in the course of in vivo incorporation? This was already described by KAKOL (1971) and observed this spring in Perry's laboratory too. The question is, will it be proved that the conditions of isolation of the heavy chain as well as the light chains and histidine peptides are not favourable for the observation of all histidine phosphates, and that is why we were able to produce phosphates of steadier bond only?

Acknowledgements

The authors are greatly indebted to Dr. L. Szabó for the total quantity of Pi^{32} and determination of radioactivity; to Mr. J. M. Wilkinson for the amino acid analysis; to Prof. Perry, Birmingham, for making it possible to perform a part of the experiments at his institute as well as for his useful comments; finally to Mrs. Bökönyi for her skilful technical assistance.

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In memoriam

Prof. I. Szörényi (1961, Magyar Tud. Akad. Orv. Tud. Oszt. Közl., *2*, 144.) was the first to observe the phosphorylated intermediar of myosin.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1–2), pp. 127–182 (1976)

VARIA

"BUDAI CSEMEGE"

Taxonomical place: *Pisum sativum* L. convar. *medullare* Alef. var. *pervicax* Alef.
Origin: Kelvedon Wonder × *Pisum sativum* L. var. *umbellatum* (L.) Sér. × Hundredfold
Beginning of breeding: 1962, Budatétény
Breeders: Dr. Kálmán Csatári-Szüts and Ágnes Baranyai, Budapest
State qualification: state certified variety, 1971
General characterization: a high yielding, drought tolerant, medium late, green-seeded marrowfat pea suitable mainly for processing purposes.

Morphological description:

Root system: medium deep, strong.

Shoot system: 50–60 cm high, stiffly erect.

Stem: solid, yellowish green, of slightly waxy surface, divided into short internodes.

Foliage: primary foliar leaves are dark green with narrow obovate blades indented at the top; normal foliar leaves are ashy green with narrow elliptic leaflets slightly serrated at the edges and cut at the apex (Mrs. Varga in litt.). A medium large tendril develops on the leaf.

Inflorescence: cluster with one or two flowers placed in the upper third of the plant (umbelliform).

Flowers: small with white corolla; semi-circular standard indented at the top, crisped at the edges and sharp toothed at the top; basis cut to horizontal; alea ovate (Mrs. Varga in litt.).

Fruit: dark green, slightly bent, pointed, medium long, narrow; the number of seeds in the pod 8—9; the upper lobe of calyx wide lanceolate; number of pods per plant generally 5—8 (Mrs. Varga in litt.).

Seed: typically "marrowfat pea"-shaped; round and flat, wrinkled; of greyish green or light yellowish green colour (dark green when young). Thousand-seed-weight: 170—180 g when fully ripe (dry) and 404—460 g when green ripe. When green the seed is of 9—11 mm diameter; 460 g of young seed are shelled from 1 kg of pods (Molnár 1971).

Biological characters:

Germination: the germinative ability of the seed is 92 per cent but only 38 per cent comes up in the field. This may be increased to 50 per cent by using Dithan as seed dressing.

Vegetative period: from sowing to flowering 63 days (total heat required: 466.9 °C); the full growth season is 95 days (total heat 850 °C); number of days until green pea harvesting 75—85 (total heat requirement: 830 °C).

Water requirement: drought tolerant plant

Resistance to disease: somewhat susceptible to Ascochyta pisi.

Farm technology requirements:

Sowing: early April is the ecologically optimum time of sowing when the temperature of the soil at a depth of 5 cm reaches 10 °C.

Soil requirement: it yields more favourably on heavy soil.

Productivity: seed yield (young) 40 q/ha shelled from some 93 q of pod. The amount of green straw is 140 q/ha; medium late (Komjáti—Molnár 1967, Molnár 1972). Since all the plants ripen at the same time it is suitable for mechanical harvesting. The variety is suitable for processing and cold-storage.

Region of cultivation: efficiently grown in all pea production regions of Hungary.

*

Prepared at the Department of Botany, University of Agrarian Sciences, Debrecen.

Gy. Mándy

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EFFECTS OF GAMMA RADIATION ON GERMINATION OF SOYBEAN SEEDS (GLYCINE MAX L.)

Soybean seeds, record very poor germination, in the field, under tropical conditions. Preliminary observations in this laboratory indicate a gradual loss of viability in this seed under storage. Some improvement in seed germination has been achieved by manipulating the storage conditions (Srivastava—Sareen 1972). Trials with growth hormones and other inorganic and organic chemicals in this laboratory have yielded no significant increase in germination. Keeping in view the radiosensitivity of seeds and their response to germination (Clark *et al.* 1968, Corneau—Szekely 1972, Fowler—MacQueen 1972, Grechushnikov—Serebrennikov 1965, Gustafsson—Simak 1958, Heaslip 1961, Johnstone—Klepinger 1967, Morgen—Johansen 1964, Saric 1961, Zolyneak *et al.* 1968), the present investigation was undertaken.

Table 1

Effect of γ radiation on germination of soybean seeds at two different stages of storage

	1st experiment Oct. 70.	2nd experiment Jan. 71.
Irradiation dose (kR)	Number of seeds germinated from sample of 50 seeds	Number of seeds germinated from sample of 75 seeds
Control	10 ± 2.8*	3 ± 1.7*
1	10 ± 2.8	11 ± 3.1
5	10 ± 2.8	+
10	18 ± 3.4	17 ± 3.6
15	+	18 ± 3.7
20	28 ± 3.5	22 ± 3.9
25	+	19 ± 3.8
30	+	17 ± 3.8
35	14 ± 3.2	18 ± 3.7
40	18 ± 3.4	+
45	20 ± 3.5	17 ± 3.6
50	19 ± 3.5	15 ± 3.5
75	20 ± 3.5	12 ± 3.0
100	9 ± 2.7	10 ± 3.0
**125	12 ± 3.0	0
150	14 ± 3.2	0
175	13 ± 3.2	0
200	8 ± 2.6	0

* Errors were calculated az 68 per cent confidence level from the expression $\sqrt{npq}$ where p is the fraction germinated, q = 1—p and n is the number of seeds sown

\+ No data because of fungal attack

** As the germination numbers are zero above 100 kR in the 2nd experiment, the data used are only upto 100 kR for the determination of an empirical relationship

Soybean seed (var. Bragg) samples were obtained from mature pods in the month of October, 1969 from the experimental area of the plant breeding department, Punjab Agricultural University, Ludhiana and stored under dry cool conditions. The healthy seeds were carefully selected to avoid any injured or wrinkled seeds. Seeds were irradiated by the Department of Atomic Energy, Govt. of India, Trombay, at doses of 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175 and 200 kR from a ^{60}Co source in the month of August, 1970. The moisture content in the seeds at the time of irradiation was about 10.5 per cent and the irradiation dose rate was 4 kR/min. Two sets of experiments were set up, one in the last week of October, 1970 and another after a gap of 75 days in the second week of January, 1971, in order to find out the changes in the germination percentage as a function of time. The sample sizes for the first and second experiments were 50 and 75 seeds respectively.

Seeds were surface sterilized with 0.2 per cent mercuric chloride for 10 minutes, thoroughly washed with distilled water and placed in glass petri dishes (4 inch diameter) between moistened circular discs of filter paper. The samples were placed in two replicates of 25 seeds each for the first experiment and three replicates of 25 seeds each for the second one. All germination studies were carried out at 25 ± 0.5 °C in the dark over a period of six days with scoring done at the end. Germination was based on the radicle having attained a length equal to that of the seed. All samples including the control were tested for germination at the same time in the same incubator in order to avoid any fluctuations in the physical conditions. Due to severe fungal attack on seeds in some of the petri dishes, three doses (15, 25 and 30 kR) in the first experiment and two doses (5 and 40 kR) in the second experiment were left out of the analysis.

Root and shoot lengths were also noted in order to find any significant change due to γ irradiation. After drying the seedlings at 80 °C for foue hours, weights of cotyledons and axes were also determined, in order to make out any possible difference from the control seeds.

Table 1 shows the number of seeds germinated at different doses in both the experiments. The usual method for testing the significance of difference between germinations in the control and treatments is to calculate

$$Z = \frac{\dfrac{g_c}{n_c} - \dfrac{g}{n}}{\sqrt{\left(\dfrac{g_c + g}{n_c + n}\right)\left[1 - \left(\dfrac{g_c + g}{n_c + n}\right)\left(\dfrac{1}{n_c} + \dfrac{1}{n}\right)\right]}}$$

where n_c = total number of seeds sown in the control

n = total number of seeds sown at each dose

g_c = number of seeds germinated in the control

g = number of seeds germinated at any particular dose.

If the calculated value of Z is more than 1.96,* then the two germinations are significantly different at 95 per cent level of confidence.

As n_c and n were equal in the present experiment $Z > 1.96$ means that

$$\frac{(g_c - g)\sqrt{2n}}{\sqrt{(g + g_c)\,[2n - (g + g_c)]}} > 1.96.$$

This equation was used to obtain two values of g (say g_1 and g_2) such that any g less than g_1 or greater than g_2 is significantly different from g_c.

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* Because of the large number of degrees of freedom, standard normal deviates were used in place of the values.

Instead of comparing the individual germination numbers with the control for testing a significant difference, the values of g_1 and g_2 were calculated. The number of seeds germinated at any dose was compared with these values of g_1 and g_2.

For the first experiment the values of g_1 and g_2 obtained are 3 and 19 respectively. As the number of seeds germinated at 20, 45, 50 and 75 kR are more than 19, the germination proportions for these doses are significantly different from the proportionate control. Amongst these four doses, germination was the highest at 20 kR.

The upper limit calculated for the second experiment is 10 (lower limit being negative having no physical significance). Hence all the germination numbers which are more than 10 in this experiment are significantly higher than the control. In this case also the germination observed at 20 kR (22/75) was the highest amongst all the doses.

The development of the empirical relationship between germination and the radiation dose is desirable because of the following reasons:

1. It is important to know the manner in which germination is affected by the radiation level to which seeds are exposed, and
2. As the γ ray doses are not continuous, from the experimental data as such it is not possible to predict the exact dose giving maximum germination.

Keeping in view the trend of variation it is evident that a curve of the type $g = AX^{B} e^{-CX}$ will be the most suitable, where g is the number of seeds germinated and X is the dose of γ ray in kR and A, B and C are constants.

The coefficients A, B and C were calculated by using the least squares method to the expression $\log g = \log (A) + B\log (X) - CX$. For the first experiment the relation is

$$g = 10.40\ X^{0.3498}\ e^{-0.01596X}. \qquad (1)$$

This relationship in terms of the percentage of germination (G) is given by

$$g = 20.80\ X^{0.3497}\ e^{-0.01596X}. \qquad (2)$$

Here only factor A has been doubled as the sample size is 50, the other constants will not be affected.

Theoretically the γ ray dose required for giving maximum germination is obtained by putting G) X = 0. The solution to the above equation gives X_{max}, the dose corresponding to the maximum germination. For the above relationship (Eq. 2) $X_{max} = B/C$. As $B = 0.3497$ and $C = 0.01596$, the value of X_{max} is 21.9 kR.

The experimental results and the curve from equation 1 are plotted in Fig. 1. In order to test the goodness of fit the χ^2 method was used. The calculated

$$\chi^2 = \left[\sum \frac{(\text{observed-expected})^2}{\text{expected}}\right]$$

is 6.80. Here the number of observation points are 8, and we have estimated 3 constants (A, B and C) from the data, the degrees of freedom for χ^2 is $8-1-3 = 4$. The value of χ^2 at 4 degrees of freedom from the table at 5 per cent level of significance is 9.49. Hence it is concluded that the curve from equation 1 appeared to give a good representation of the experimental observations.

Similarly the empirical relationship for the second experiment was obtained as

$$g = 10.86\ X^{0.2857}\ e^{-0.01448X}. \qquad (3)$$

But the comparison of this curve with the first one can only be done in precentage of germination, as the sample sizes (number of seeds) are different in the two cases. The percentage of germination, G in the second experiment is given by

$$G = 14.5\, X^{0.2857}\, e^{-0.01448X} \qquad (4)$$

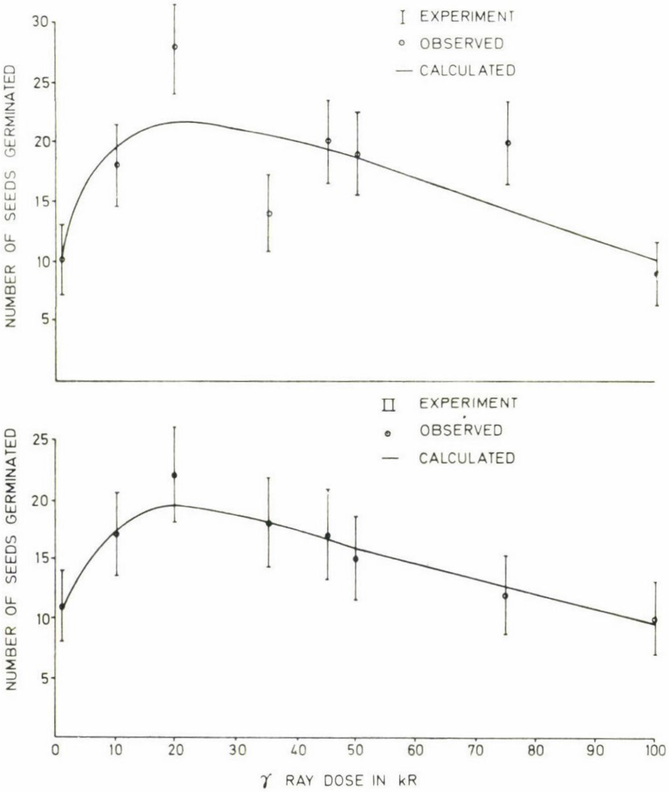

Fig. 1. The points $\overset{\top}{\underset{\bot}{\odot}}$ represent the number of seeds germinated as a function of the gamma ray dose in both the experiments. The curves are least square fits to the experimental data. The sample sizes were 50 and 75 seeds in the 1st and 2nd experiment respectively

The maximum of the curve is obtained at the γ ray dose of $X_{\max} = B/C = 19.8$ kR. The experimental results and the calculated curve from equation 3 are shown in Fig. 1. Here also χ^2 was applied to test the goodness of fit of the empirical curve. The calculated value of χ^2 is 0.63 and the value from the table is 9.49. Hence the empirical curve appeared to be a very good representation of the experimental points.

As it appears from equations 1 and 3 i.e. from the shape of the empirical curves drawn, the two regression functions are the same apart from a change in the constant A. To test this hypothesis an 'F' test (RAO 1952) was applied. The variance ratio obtained is 0.22. The value of F from the table at 2 and 10 degrees of freedom is 4.10 at 5 per cent level of significance. The value is, therefore, not significant and the test furnishes no evidence against the hypothesis that 'The + two regression functions are the same apart from a change in the constant A'.

As the shapes of the two regressions are not found to be different, the pooled estimates of B and C were obtained as 0.3198 and 0.01514 respectively. Hence germination can be represented as a function of the χ ray dose by the expression.

$$G = A\,X^{0.32}\,e^{-0,015X}$$

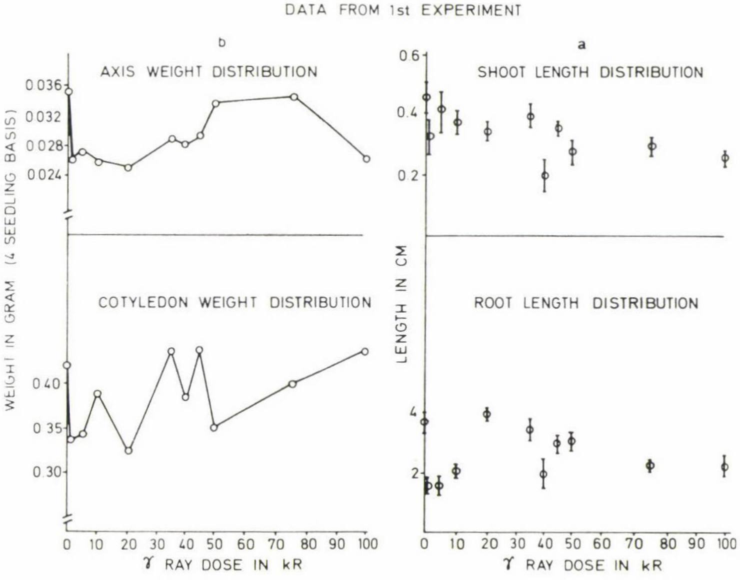

Fig. 2. (a) Represents the distribution of shoot and root lengths as a function of the gamma ray dose; (b) Represents the distribution of axis and cotyledon weights as a function of the gamma ray dose

This relation does not hold at $X = 0$ as the radiation doser in the experiment started from 1 kR. Also in practice X is never zero, because of the χ ray components of cosmic rays. The value obtained for $X_{\max}$ from this equation is 21.3 kR. From the above relationship it is evident that the germination is increased by about 25 per cent above the control at the dose of 20 kR.

The distribution of root and shoot lengths for the various doses of χ rays is presented in Fig. 2(a). The distribution does not show any significant change in length at any dose. Fig. 2(b) shows the weight distribution of cotyledons and axes. Here also no appreciable change is observed.

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STUDIES ON THE ACTION OF SOME GROWTH RETARDANTS ON COTTON PLANTS

As cotton is considered to be one of the most important field crops in the world, it is of prime importance to study the effect of the prevailing factors which govern its growth and yield as the effect of plant growth substances particularly growth retardants and inhibitors. Addicott *et al.* (1964) reported that abscisic acid (ABA) has a marked effect on inducing senescence in cotton plants, and hence affects the yield by shortening their growth period. Maleic hydrazide (MH) also has been studied extensively as an inhibitor of plant development and as an antimitotic agent (Crafts 1964, Hoffman—Parups 1964, Kihlman 1965). Recently, Nooden (1969) reported that MH inhibits corn root elongation through an effect on cell division apparently without inhibiting cell enlargement. He concluded that the data did not support the theory that MH acts by inhibiting the synthesis of or competing with some simple metabolite or hormone.

In 1964, Cathey reported that the growth regulator (2-chloroethyl)-trimethyl ammonium chloride (CCC) is one of a group of regulators known as growth retardants. The primary effect of these retardants on most plants is the inhibition of stem elongation.

In a glass house study, Thomas (1964), reported that CCC reduced the elongation of the main stem and fruiting branches. This reduction was accompanied by a moderate reduction in the rate of flowering, in boll set and seed cotton production. Mathar (1963) showed that flowering in cotton plants was hastened by 20 ppm napthalene-acetic acid (NAA) and delayed by 20 ppm B-naphthoxyacetic acid (NOA), 30 ppm indolylacetic acid (IAA) and 5 ppm 2,4-dichlorophenoxyacetic acid (2.4-D). More flowers were formed compared to the untreated control with IAA and 2,4-D and less with NAA and NOA. Following such auxin treatments, final flower retention was higher than in the controls. Flowering was also accelerated by the anti-auxin triiodobenzoic acid (TIBA) (40 ppm), and 20 ppm trans-cinnamic acid (trans-CA), but while trans-CA increased flower production and retention, TIBA reduced flower production and had little effect on retention. **Bhardwaj—Santhanam** (1964) reported that bud and boll shedding were reduced by NAA (the former mainly by 20 ppm). NAA extended the first flowering peak by about 10 days, increased flowering at the second peak and hastened maturity. The yield of seed cotton per boll was increased especially by spraying at the bud formation stage. However, no significant effect of gibberellin on the root system of cotton was observed by **Agakisiev** (1964) whereas pre-sowing soaking of cotton seeds in gibberellin solution stimulated the growth of shoots during the initial period of plant development only.

Following these results, it was found of considerable importance to study the effects of some growth regulators as MH, CCC, B_{995} and Amo $_{1618}$ on the growth and yield of cotton cv. "Ashmoni" widely grown in upper Egypt.

This experiment was planned in a randomized complete block design with 4 replicates. It was carried out for two seasons. The experimental unit was 1/400 acre (4 × 5 meters) in area. It contained five rows with a distance of 20 cm between hills and 10—15 seeds per hill, thinned to two seed seedlings after recording the germination percentage. Cotton cv. "Ashmoni" was used in this investigation. The seeds were soaked for 24 h in one of the following solutions before planting: (i) distilled water to represent the control, and (ii) aqueous solution containing one of the following growth substances: CCC ((2-chloroethyl) trimethyl ammonium chloride), Amo $_{1618}$ (4-hydroxy-5-isopropyl-2-methyl phenyl trimethyl ammonium chloride, 1-piperidine carboxylate), B_{995} (N-dimethylamino succinamic acid), or MH (1,2-dihydro-3,6-pyridazinedione), at a concentration of 50 and 100 ppm each. At the end of the soaking period, the seeds were washed thoroughly with distilled water to remove any adhering chemicals.

After planting, all agricultural practices were carried out as usual. In order to estimate plant growth and yield, the following characteristics were recorded: (i) number of both vegetative and fruiting branches per plant for the ten plant samples from each plot, (ii) total number of bolls per plant for also 10 plant samples, and (iii) yield per plot in kilograms.

The statistical analyses of the data were performed and Duncan's multiple range test was used to compare the effect of the various treatments.

Effects on number of vegetative branches per plant. The results presented in Table 1 for the two seasons show that although 50 and 100 ppm MH in both seasons increased the number of vegetative branches per plant compared to other growth retardants and the controls, yet the Duncan's multiple range test revealed no significant differences between any two means in both seasons.

Effects on number of fruiting branches per plant. The data given in Table 2 for the two seasons show that 50 and 100 ppm MH for the first and second seasons respectively, increased the number of fruiting branches per plant compared to CCC, B_{995}, Amo $_{1618}$ and the controls. However, no significant differences were detected between MH and CCC, B_{995}, or Amo $_{1618}$ means at the concentration of 50 ppm, while the differences between the effect of 50 and 100 ppm MH were statistically significant for the first season. In the second season, although 100 ppm MH resulted in a higher number of fruiting branches than 50 ppm MH and other treatments including the controls, no significant differences were detected between the effect of 100 and 50 ppm MH.

Effects on number of bolls per plant. The data in Table 3 reveal also that both MH concentrations of either 50 or 100 ppm for the second or first seasons respectively, resulted in an increase in the number of bolls per plant compared to other growth retardants and the controls. However, although the differences between 50 and 100 ppm MH were found to be statistically significant in the first season, yet no significant differences were detected between the effect of the two concentrations in the second season. On the other hand, the results indicated that there were significant differences between 50 ppm MH and 50 and 100 ppm B_{995} and Amo_{1618} for first season, whereas there were no significant differences between 50 and 100 ppm MH, 50 ppm CCC, 50 ppm Amo $_{1618}$, and 50 ppm B_{995} for the second season.

Table 1

Possible comparisons among treatment means for the effect of MH and some growth retardants on the number of vegetative branches per plant for two growing seasons

First season:									
Chemicals	CCC	Amo_{1618}	B_{995}	CCC	Control	MH	B_{995}	Amo_{1618}	MH
Conc. ppm	100	50	50	50		50	100	100	100
Means	4.40	4.60	4.60	4.90	4.95	5.40	5.50	5.55	6.10
	a	a	a	a	a	a	a	a	a
LSR(0.05)	4.90	4.32	4.41	4.51	4.61	4.64	4.68	4.74	
Second season:									
Chemicals	B_{995}	Control	CCC	MH	CCC	B_{995}	Amo_{1618}	Amo_{1618}	MH
Conc. ppm	100		100	100	50	50	100	50	50
Means	4.02	4.27	4.27	4.27	4.35	5.55	5.70	5.77	6.82
	a	a	a	a	a	a	a	a	a
LSR(0.05)	3.78	3.66	4.07	4.17	4.23	4.27	4.33	4.34	

Effects on yield per plot. The yield data presented in Table 4 show that 50 ppm B_{995} increased the yield per plot in kilograms in both seasons. On the other hand, MH which increased the number of vegetative branches, fruiting branches, bolls per plant failed to show a significant effect on the yield in both seasons. However, although 50 ppm MH resulted in a marked increase in the first season's yield, it was significantly lower than the increase due to 50 ppm B_{995} which produced the highest yield among all treatments.

In 1965, Cleland (CLELAND 1965) concluded that there is more than one mode of action of MH, and that its actions may differ according to the species, the tissue and response studied, while NOODEN (1969) reported that MH inhibits cell division apparently without inhibiting cell enlargement. He concluded that these data did not support the theory that MH acts by inhibiting the synthesis of or competing with some simple metabolite or hormone. On the basis of these reports, the mode of actions of MH and the growth retardants CCC, B_{995}, Amo_{1618} on "Ashmoni" cotton will be discussed.

Although the results showed no significant differences between the effect of MH concentrations, growth retardants, and controls on the number of vegetative branches per plant, yet MH at 100 and 50 ppm in the first and second seasons respectively, resulted in increasing the number of vegetative branches per plant compared to the growth retardants and the controls. The results also showed that 50 ppm of MH resulted in increasing the number of bolls per plant in both seasons.

Following these results, the increase in the number of either vegetative or fruiting branches and the number of bolls per plant as a result of MH treatments compared to the growth retardants was expected, since it is well known that MH inhibits cell division in plants at the appropriate concentrations without affecting cell enlargement (KIHLMAN 1965, NOODEN 1969). This indicates that MH has no effect on the action of the endogenous gibberellin-like substances which are responsible mainly in inducing cell enlargement and hence the growth of any plant part. On the other hand, the physiological response in growth induced by MH treatments would not be obtained by any of the growth retardants, as the latters have an antigonistic effect on the gibberellin biosynthesis in plants (EL-ANTABLY 1970) and hence the growth of any plants treated with these retardants in restricted as has been shown in this work.

Concerning the effect of B_{995}, although 50 ppm resulted in decreases in the vegetative and fruiting branches and the number of bolls per plant, this concentration was found to be able to increase the yield per plot in both seasons (Table 4) compared to both MH concentrations and other growth retardants. However, this retarding effect seems to be due to the blocking of gibberellin biosynthesis, followed by a reduction in the growth of the treated plants. On the other hand, the retarding effect was found to be removed after a short time, during which the plants seemed to be able to synthesise new amounts of gibberellins enough for inducing both cell enlargement and division. Therefore, more yield was obtained as an effect of B_{995} which resulted in a reduction in vegetative growth compared to other growth regulators including MH which failed to produce higher yields. This also could be related to the lasting effect of MH in inhibiting cell enlargement, other simple metabolic process, and the competition with the endogenous hormones as the gibberellings which play an important role in flowering and fruiting processes. However, the variability in the results of the effect of B_{995} and other retardants as CCC and Amo_{1618} is in agreement with the finding of CLELAND (1965).

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Table 2

All possible comparisons among treatment means for the effect of MH and some growth retardants on the number of fruiting branches per plant for two growing seasons*

First season:									
Chemicals	B_{995}	Amo_{1618}	MH	CCC	Control	Amo_{1618}	B_{995}	CCC	MH
Conc. ppm	100	100	100	100		50	50	50	50
Means	14.27	16.27	16.50	17.62	18.92	19.80	20.25	20.35	20.38
	a	ab	abc	bcd	bcd	cd	d	d	d
LSR (0.05)	3.02	3.18	3.26	3.33	3.39	3.43	3.46	3.49	
Second season									
Chemicals	B_{995}	B_{995}	CCC	MH	CCC	Control	Amo_{1618}	Amo_{1618}	MH
Conc. ppm	50	100	50	50	100		50	100	100
Means	17.10	18.40	18.67	18.90	19.55	19.70	19.70	19.80	20.15
	a	ab	ab	ab	ab	ab	ab	b	b
LSR (0.05)	2.31	2.43	2.49	2.54	2.59	2.61	2.64	2.66	

* The difference between any two means having the same letters or the same group of letters is not significant (Duncan's multiple range test)

Table 3

All possible comparison between treatment means for the effect of MH and some growth retardants on the number of bolls per plant for two growing seasons*

First seasons:									
Chemicals	B_{995}	MH	Control	Amo$_{1618}$	Amo$_{1618}$	CCC	B_{995}	CCC	MH
Conc. ppm	100	100		100	50	100	50	50	50
Means	25.12	27.42	29.57	29.67	29.85	33.32	41.70	43.90	50.85
	a	ab	ab	ab	ab	b	c	cd	d
LSR (0.05)	6.13	6.46	6.62	6.77	6.91	6.97	7.02	7.10	
Second season									
Chemicals	Control	B_{995}	B_{995}	Amo$_{1618}$	Amo$_{1618}$	CCC	CCC	MH	MH
Conc. ppm		100	50	50	100	100	50	100	50
Means	31.45	38.50	40.50	41.10	42.20	42.50	42.72	43.90	46.20
	a	ab	bc	bc	bc	bc	bc	bc	c
LSR (0.05)	6.00	6.29	6.48	6.60	6.72	6.81	6.87	6.91	

* The difference between any two means having the same letters or the same group of letters is not significant. (Duncan's multiple range test).

Table 4

All possible comparison among treatment means for the effect of MH and some other growth retardants on the yield per plot in kilograms for two growing seasons*

First season:									
Chemicals	Amo_{1618}	CCC	Amo_{1618}	B_{995}	MH	Control	CCC	MH	B_{995}
Conc. ppm	50	100	100	100	100		50	50	50
Means	5.75	6.15	6.20	6.70	7.90	8.35	8.52	9.20	12.50
	a	ab	abc	abc	abc	abc	bc	c	d
LSR (0.05)	2.39	2.52	2.58	2.64	2.69	2.71	2.74	2.76	
Second season:									
Chemicals	MH	MH	Amo_{1618}	Control	CCC	CCC	Amo_{1618}	B_{995}	B_{995}
Conc. ppm	100	50	100		100	50	50	100	50
Means	7.86	8.47	8.47	8.72	9.22	9.22	9.23	9.31	9.34
	a	ab	bc	bcd	bcd	bcd	bcd	cd	d
LSR (0.05)	0.730	0.780	0.790	0.810	0.820	0.830	0.840	0.844	

* The difference between any two means which having the same letters or the same group of letters is not significant. (Duncan's multiple range test)

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EARLY DEVELOPMENT AND SPATIAL ARRANGEMENT OF THE VASCULAR BUNDLE SYSTEM IN SOME SPECIES OF CUCURBITACEAE

The internal organization, and partly the histogeny of the best known species and cultivated varieties of *Cucurbitaceae* were studied by many researchers in the second half of the last century. It was then that the bicollateral vascular bundle, the type of vascular bundle characteristic of the family was discovered (Hartig 1854). In this — as it is known — an external and an internal cambium differentiate. The function of the external cambium is well known, but as to the development and function of the internal cambium literary data are available but sporadically. Therefore we considered it necessary to subject the development and functioning of the internal cambium in the vascular bundles of *Cucurbitaceae* to a closer examination.

Of the relevant literature we refer mainly to the works of Hartig (1854), Mohl (1855), Nägeli (1861), Sanio (1864), de Bary (1877), from whom the name originates, Bertrand (1880), Lotar (1881), Fischer (1885), Hérail (1885), Lamounette (1890), Baranetsky (1900), Faber (1904), further of Holroyd (1924), Hayward (1938), Whiting (1938), Sárkány (1943), Esau (1953), Kaussmann (1963), Lott (1970).

In the course of our investigations we used microscope and partly electron microscope methods, and dealt with the following species of the family:

Detailed investigations were made with *Cucurbita maxima*, then at certain stages with *Cucurbita pepo*, *Citrullus lanatus*, *Lagenaria vulgaris*, *Luffa cylindrica*, *Cucumis melo* and

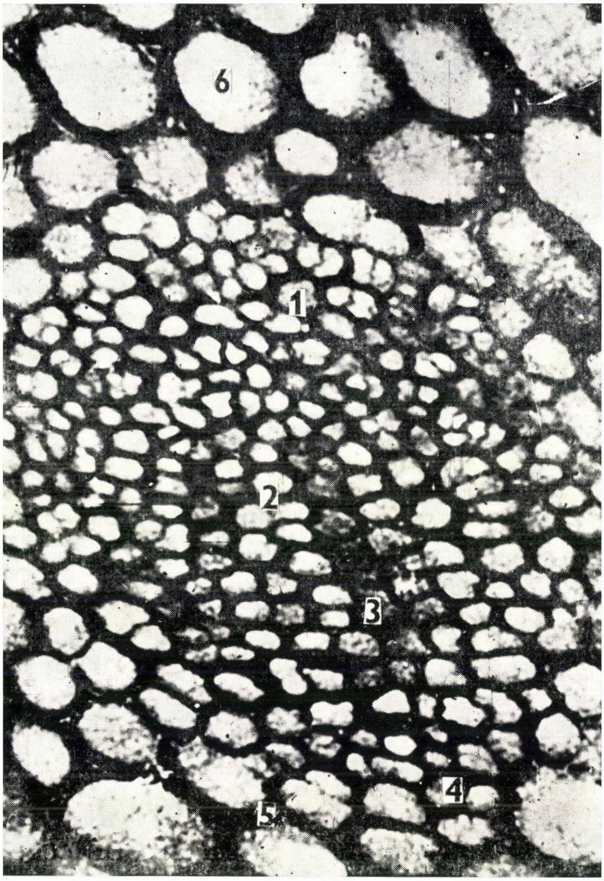

Fig. 1. Hypocotyl part from a *Cucurbita maxima* seed swelled for half an hour; in the determinate heterogeneous procambium bundle four zones can be distinguished (1 = future phloem, 2 = future outer cambium, 3 = future outer xylem, 4 = common meristem of the future inner phloem and inner cambium, 5 = future fiber elements, 6 = determinate ground meristem) (Cross section 3.2 oc. × 20 obj.)

Cucumis sativus. We started with a comparative analysis of the embryo in the swollen seed of each of the above species, and in it first of all of the determinated procambial system. We wished to find out whether at that early stage there is any difference between the examined species in the organization of the hypocotyl and cotyledons, and if so, what its extent and regularity is. In this respect we sought to get information first of all about the determinate character and functioning of the vascular system, about its numerical conditions and spatial arrangement.

After this we followed the differentiation stages of the vascular system in 4, 7—8, 10 and 14—20 days old seedlings, then in the subapical shoot axis of several months old plants. Beside this, we studied with special attention the development of the inner phloem and inner cambium of the bicollateral vascular bundle in the hypocotyl and the different internodes, as well as the mono- and dipleuric function of the inner cambium, producing phloem and xylem elements.

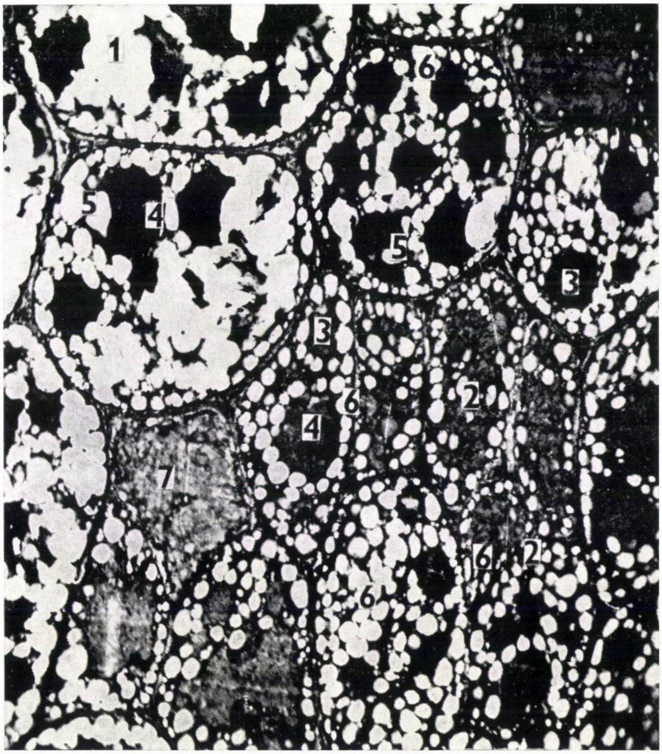

Fig. 2. Cotyledon part of a *Cucurbita maxima* embryo swelled for half an hour, ultrathin section (1 = determinate ground meristem, 2 = determinate procambium, 3 = heterogeneous aleuron grain, 4 = crystalloid, 5 = globoid, 6 = lipid bodies, 7 = soluble aleuron grains and lipid bodies). (EM photo: 3000 ×)

The present paper gives account of a small detail of our work done so far, namely of investigations and results concerning the development and spatial arrangement of the determinate procambial vascular system in the hypocotyl of the embryo. We note that we call the procambial bundles in the embryo of the nearly dormant seed "determinate" because even with a light microscope (Fig. 1), but still more so when studying the ultrathin sections by an electron microscope (Fig. 2) we can clearly see that in the cells, in the originally typical meristemic elements, well defined stored nutrients (heterogeneous aleuron grains and lipid bodies) prevail over the living plasm content (Fig. 2), and as a consequence the value of the so called P/R ratio (SÁRKÁNY *et al.* 1970, SÁRKÁNY 1973) is rather low, about 0.2—0.4.

Irrespective of the fact that at the determinate procambial stage the future vascular system of the hypocotyl performs a temporary function of storing, the number, spatial arrangement and interconnection of the vascular bundles in the hypocotyl, that is the quantitative trend of the bundle network can be considered as final and — according to our observations — characteristic of the species studied by us. Namely we found that the simplest type of vascular bundle network, the one consisting of the lowest number of main bundles was represented by the *Cucumis sativus* L. (Fig. 3) and the closely related *Cucumis melo* L. (Fig. 4). In both of them the four uniform determinate procambium bundles of the radicle pass over upwards through a procambium ring at the border of the radicle and hypocotyl to a total of four bundles

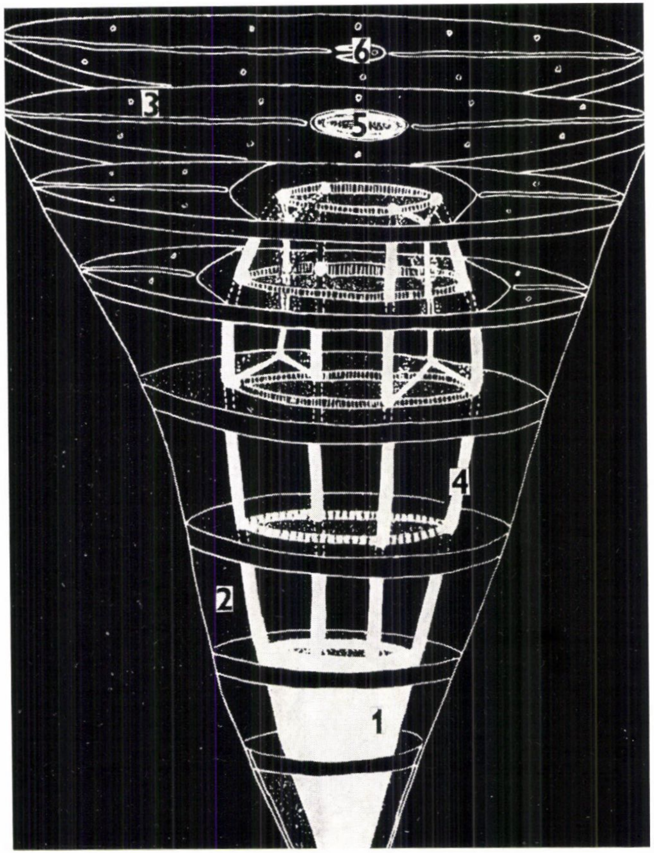

Fig. 3. Graphic illustration of the determinate procambium bundle system of an embryo prepared from a *Cucumis sativus* seed swelled for half an hour (1 = radicle, 2 = hypocotyl, 3 = cotyledon, 5 = epicotyl, 6 = first foliage leaf primordium). (cca. 100×)

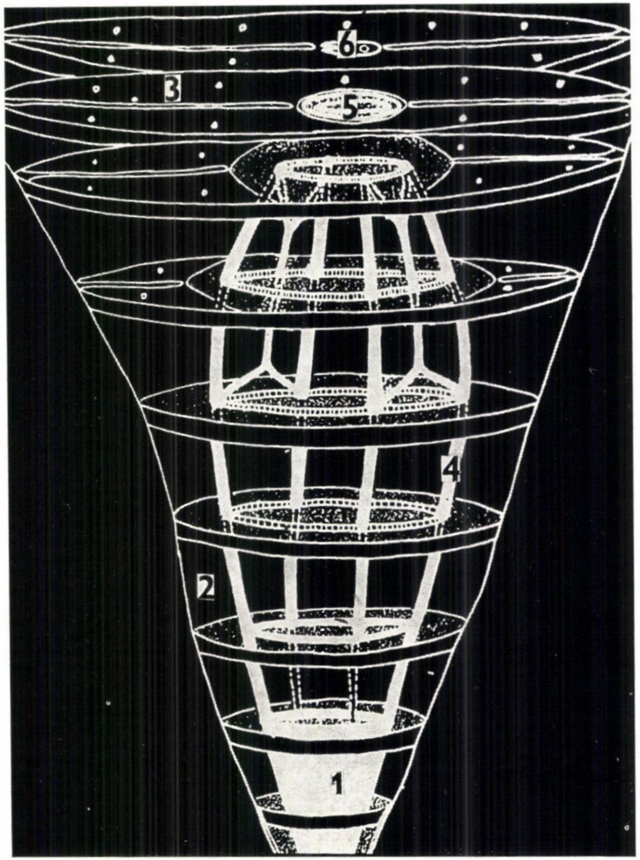

Fig. 4. Sketch of the spatial arrangement of the determinate procambium bundle system (4) of an embryo prepared from a *Cucumis melo* seeds welled for half an hour (1 = radicle, 2 = hypocotyl, 3 = cotyledon, 5 = epicotyl, 6 = first foliage leaf primordium). (cca. 100×)

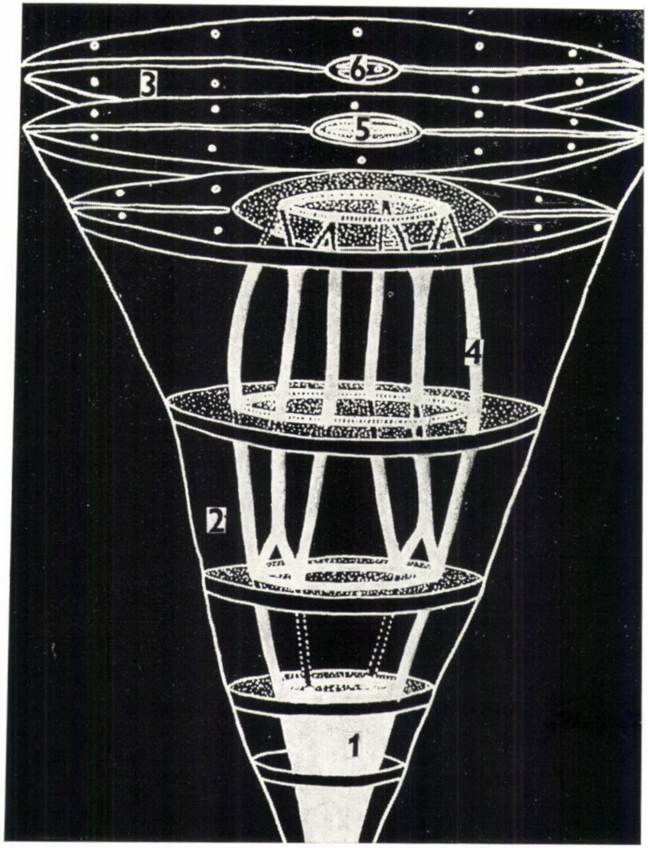

Fig. 5. Sketchy graphic illustration of the spatial arrangement of the determinate procambium bundle system (4) of an embryo dissected from a *Luffa cylindrica* seed swelled for half an hour (1 = radicle, 2 = hypocotyl, 3 = cotyledon, 5 = epicotyl, 6 = first foliage leaf primordium). (cca. 50 ×)

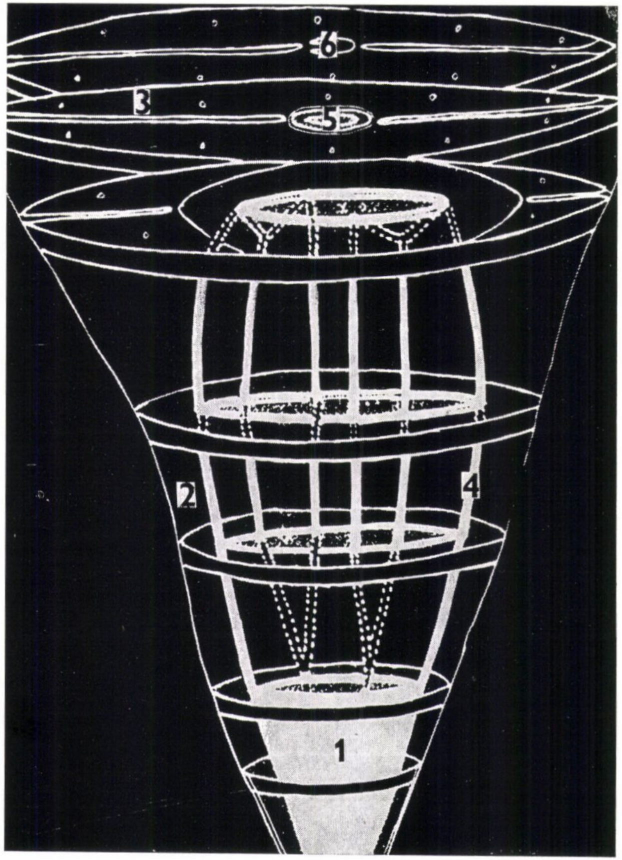

Fig. 6. Sketch of the spatial arrangement of the determinate procambium bundle system (4) of an embryo dissected from a *Lagenaria siceraria* seed swelled for half an hour (1 = radicle, 2 = hypocotyl, 3 = cotyledon, 5 = epicotyl, 6 = first foliage leaf primordium). (cca. 40 ×)

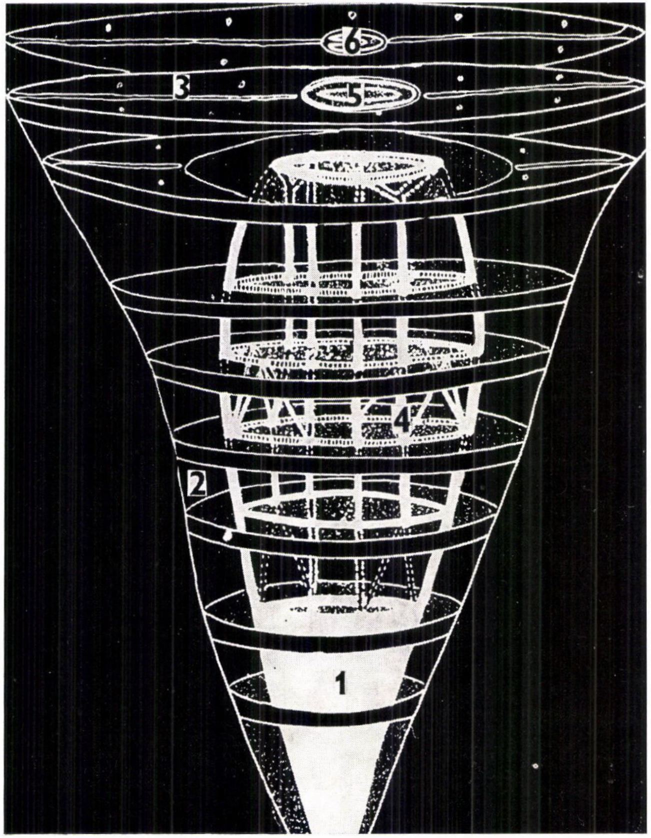

Fig. 7. Sketch of the spatial arrangement of the determinate procambium bundle system (4) of an embryo dissected from a *Citrullus lanatus* seed swelled for half an hour (1 = radicle, 2 = hypocotyl, 3 = cotyledon, 5 = epicotyl, 6 = first foliage leaf primordium). (cca. 70 ×)

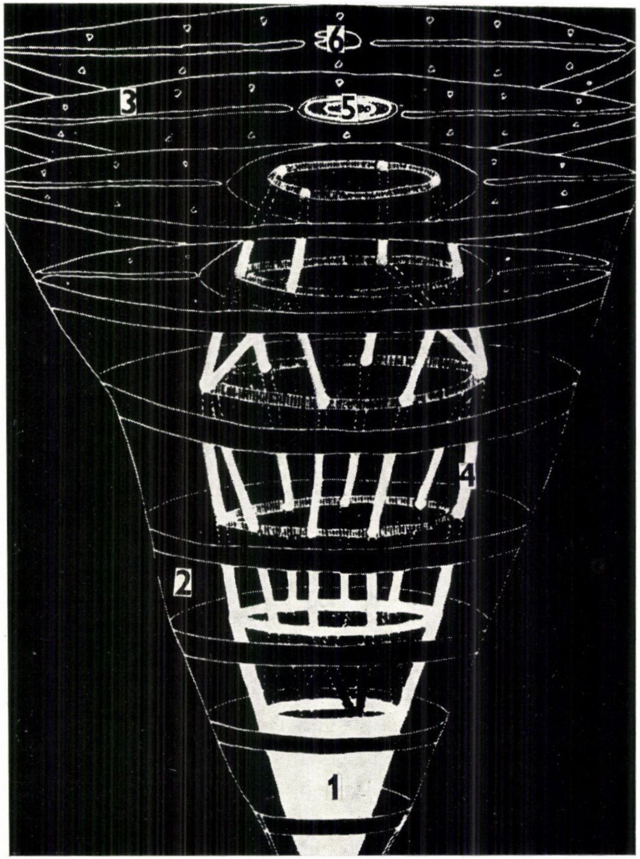

Fig. 8. Sketch of the spatial arrangement of the determinate procambium bundle system (4) of an embryo dissected from a *Cucurbita pepo* seed swelled for half an hour (1 = radicle, 2 = hypocotyl, 3 = cotyledon, 5 = epicotyl, 6 = first foliage leaf primordium). (cca. 50 ×)

in the hypocotyl; in the upper third part of the hypocotyl the number of bundles rises to six owing to the branching off of two bundles, but in the nodes of the cotyledons, through melting, the characteristic four determinate procambium bundles can be seen again; two of them pass over to one, two to the other cotyledon. In contrast to the *Cucumis* species, the most differentiated bundle supply can be observed in the hypocotyl of the embryo of *Cucurbita maxima* (Fig. 9). Here in the nodes of the cotyledons four determinate procambium bundles can be likewise found, but further down, through gradual branching off eight, then dominantly ten main bundles of equal quantitative value can be seen; towards the radicle they melt again into eight, then in the hypocotyl base into four determinate procambium bundles, characteristically of each of the examined species. The bundle network of the other species can be placed between the above two extremities, namely: *Cucurbita pepo* (Fig. 8) and *Citrullus* (Fig. 7) with maximum 6 dominant and 2 or 4 transitional (smaller), *Lagenaria* (Fig. 6) with exclusively 6 while *Luffa* (Fig. 5) in the lower level of the hypocotyl — similarly to the *Cucumis* species — with 4, but maximum 6 dominant determinate procambium bundles.

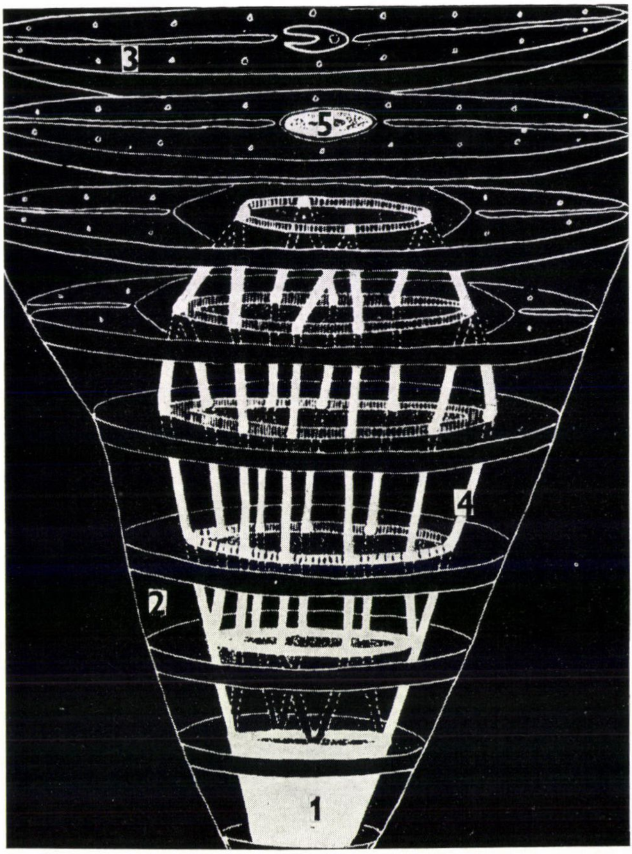

Fig. 9. Sketch of the spatial arrangement of the determinate procambium bundle system (4) of an embryo dissected from a *Cucurbita maxima* seed swelled for half an hour (1 = radicle, 2 = hypocotyl, 3 = cotyledon, 5 = epicotyl, 6 = first foliage leaf primordium). (cca. 50 ×)

On the above basis, we have arrived at the conclusion that the conditions of bundles developing in the hypocotyl of the embryo are hereditary. So, on the one hand, they are of taxonomic value and can thus be used in plant histological diagnostics, on the other hand they suggest phylogenetic correlations too. There are, namely, numerous examples of the increasing "number" of certain organs, organ parts or other quantitatively measurable properties indicating a progression in many cases. In this sense, on the basis of the prevailing number of bundles, the following phylogenetic progression can — in our opinion — be established: *Cucumis* species — *Luffa* — *Lagenaria*, *Citrullus* — *Cucurbita pepo* — *Cucurbita maxima*. This phylogenetic succession is, otherwise, supported by the quantitative trend of the veins of cotyledons.

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EFFECT OF SUBMERGENCE ON THE CHEMICAL CHANGES IN DIFFERENT RICE SOILS. II. KINETICS OF P, FE AND MN

In an earlier communication (Mohanty—Patnaik 1974), the kinetics of pH, Eh, C and N in different rice soils as a function of the time of submergence were reported as quantified general equations. This paper deals with the kinetics of P, Fe and Mn, whose transformations are very much interrelated in these soils as a function of the time of submergence.

The details of soil studies and the techniques of extraction of the incubated soils have already been reported (Mohanty—Patnaik 1974). Fe and Mn in the NaCl extract were analysed by thiocyanate and periodate methods respectively (Snell—Snell 1949).

The change in water soluble P was determined separately at specific intervals, shaking the contents of the incubated samples at 1 : 1 soil water ratio for 30 minutes and filtering. Phosphorus in the water extract was estimated colorimetrically (Jackson 1958).

The data were processed in IBM 1620 computer to fit the kinetics in linear, quadratic, logarithmic and exponential functions.

In discussing the results obtained in these investigations, data in respect of the values of the parameters studied have not been presented to save space but have been stated in the text. Of the four functions fitted for each parameter, the one with the highest R^2 value has been selected and only summary tables presented on these prediction equations giving their nature "*a*", "*b*", "*c*" and R^2 values, where "*Y*" is the value of the parameter studied at the time of submergence "*t*" days, "*a*" is constant and "*b*" and "*c*" are regression coefficients.

Change in water soluble phosphorus. Out of 20 soils studied, 9 soils showed quadratic type of change as a function of time of submergence (Table 1). There was a decrease in water soluble P during the first 20 days followed by an increase between 20—50 days of submergence, after which there was a slight decrease. These soils were low to medium in clay content, and medium to high in organic carbon. Soils having high iron and clay content showed logarithmic type of change in water soluble phosphorus. In these soils, the amount of phosphorus coming into the soil solution decreased gradually during the first 20 days, increased during the next ten days and again decreased. On the other hand, soil having medium clay content and medium to high carbon content showed exponential type of change in water soluble phosphorus. In these soils the water soluble phosphorus showed slight increase between 20—30 days of submergence. Soils having initial pH 4.7 to 5.5, gave higher amount of water soluble P compared to 5.5, gave higher amount of water soluble P compared to other soils.

Change in available iron. It can be seen from Table 2 that 16 out of 20 soils used in the investigation showed exponential type of change in available Fe. In these soils, the maximum amount of available Fe was obtained between 20—50 days of submergence after which there was a gradual decrease. Though the soils differed widely in their physico-chemical properties, acid soil having pH less than 5 and low cation exchange capacity (8.5 to 11.5 m.e./100 gm soil) showed the highest amount of available iron and the magnitude of increase in these soils was very high. The amount of available Fe at 70 days of submergence was found to be more than the initial content of the soil. But in soils with pH more than 6.7 and high CEC, the change in available Fe was very slow.

Soils 3, 17 and 18 showed logarithmic type of change in available iron, where the peak was obtained between 10—20 days of submergence. The magnitude of change of available Fe in these soils as a function of time was, however, not very marked.

Change in available manganese. Eighteen soils having low to moderate total Mn and high Fe showed exponential type of change, where available Mn increased very rapidly and gave a peak at 20 days of submergence and then decreased gradually. The values obtained on 70th day of submergence were much more than the initial values. Soils having low total Mn content showed lower availability as compared to the soils having moderate contents.

Table 1

Equations for change in water soluble phosphorus with time of submergence in different soils

Soil No. (location and soil type)	Equations best fitted	"a"	"b"	"c"	R² %
1 (Bhubaneswar, laterite)	$Y = a + bt + ct^2$	1.30	−0.57	0.07	76.69
3 (Pattambi, laterite)	-do-	0.16	0.08	−0.02	30.50
8 (Gamharipalli, red loam)	-do-	0.30	0.05	−0.006	19.54
9 (Chiplima, red loam)	-do-	0.05	0.04	−0.004	14.67
10 (Bargarh, red loam)	-do-	0.15	−0.02	0.002	15.89
11 (Barpali, red loam)	-do-	0.18	0.03	−0.004	22.54
12 (Cuttack, alluvial)	-do-	1.44	−0.35	0.03	39.31
14 (Kujang, alluvial)	-do-	0.08	0.07	−0.01	37.66
18 (Palur, black)	-do-	0.14	0.002	−0.004	34.19
2 (Sukinda, laterite)	$Y = a + b \ln (t + 1) + c [\ln(t + 1)]^2$	0.71	0.33	−0.24	21.88
15 (Kendrapara, alluvial)	-do-	1.31	−1.86	0.76	71.75
16 (Bolangir, black)	-do-	0.06	0.03	−0.03	90.29
17 (Arkhabahali black)	-do-	0.08	0.02	−0.006	11.04
4 (Mysore, laterite)	$\ln Y = a + b \ln (t + 1) + c[\ln (t + 1)]^2$	−0.006	0.54	−0.27	79.73
5 (Berhampur, red loam)	-do-	1.02	−0.34	0.05	30.30
6 (Chakuli, red loam)	-do-	0.06	0.006	−0.02	86.94
7 (Parmanpur, red loam)	-do-	0.05	0.06	−0.04	85.43
13 (Sakhigopal, alluvial)	-do-	0.87	−0.58	0.22	19.05
19 (Nellore, black)	-do-	0.30	0.03	−0.09	49.83
20 (Keshpur, saline)	-do-	0.10	0.79	−0.42	40.65

Soils 8 and 16 having high total Mn and moderate total Fe showed logarithmic type of changes in available manganese. In these soils there was a marked increase in available Mn during the first 10 days, followed by a gradual increase upto 20 days after which there was a slow decrease upto 70 days, this value being higher than the initial value.

Table 2

Equations for change in NaCl extractable iron with time of submergence in different soils

Soil No.	Equations best fitted	"a"	"b"	"c"	R^2 %
6	$Y = a + bt + ct^2$	19.70	0.36	-0.36	38.00
3	$Y = a + b \ln (t + 1) + c[\ln (t + 1)]^2$	13.04	-5.39	1.31	32.42
17	*-do-*	0.06	0.63	-0.33	40.81
18	*-do-*	7.27	6.77	-3.12	7.80
1	$\ln Y = a + b \ln (t + 1) + c[\ln (t + 1)]^2$	2.69	3.16	-0.90	98.17
2	*-do-*	2.62	2.22	-0.96	81.01
4	*-do-*	3.48	0.26	-0.44	78.50
5	*-do-*	4.41	-0.09	0.15	49.66
7	*-do-*	2.80	1.77	-0.65	69.00
8	*-do-*	2.58	2.36	-0.99	48.93
9	*-do-*	3.32	-0.06	-0.29	90.12
10	*-do-*	4.45	0.29	-0.43	52.00
11	*-do-*	4.35	-0.64	0.002	57.59
12	*-do-*	1.79	2.62	-1.01	67.09
13	*-do-*	0.16	3.37	1.09	88.60
14	*-do-*	1.90	0.73	-0.28	81.59
15	*-do-*	1.21	4.24	-1.40	96.96
16	*-do-*	0.17	1.84	-0.80	53.29
19	*-do-*	0.81	1.47	-0.99	56.25
20	*-do-*	1.05	0.87	-0.42	64.39

The increase in phosphorus during the initial period of submergence might be due to the reduction of insoluble ferric phosphate to more soluble ferrous phosphate (Chiang 1963, Islam–Elahi 1954, Mandal 1964) and the release of occluded phosphate by the reduction of hydrated ferric oxide coatings (Chang—Jackson 1958). The decrease in phosphorus at later periods might be attributed to precipitation, change in pH, immobilization due to microbial activities and isomorphous replacements (Annonymous 1963, 1964, Chiang 1963, Ponnamperuma 1964).

The increase in availability of Fe was chiefly due to the chemical reduction of ferric iron in the absence of a higher level of oxidative compounds. The subsequent decrease in iron availability was probably due to the formation of unstable bicarbonate in the presence of sufficient carbon dioxide which was further precipitated as sulphides in the absence of oxygen (Mandal 1961).

The increase in Mn availability due to submergence might have been due to reduction of manganic compounds by serving as electron acceptors in the respiration of micro-organisms in the absence of a higher level of oxidative compounds produced during the anaerobic decomposition of soil organic matter and also due to the reduction of the reducible Mn fraction (Mann—Quastel 1946, Turner 1967). Subsequent decrease in manganese availability could be due to the precipitation of manganese as manganese carbonate (Anonymous 1964).

Table 3

Equations for change in NaCl extractable manganese with time of submergence in different soils

Soil No.	Equations best fitted	"a"	"b"	"c"	R² %
8	$Y = a + b \ln (t + 1) + c[\ln (t + 1)]^2$	112.90	618.82	228.69	98.11
16	-do-	33.38	173.83	−66.34	96.89
1	$\ln Y = a + b \ln (t + 1) + c[\ln (t + 1)]^2$	0.07	5.65	−1.92	98.51
2	-do-	1.80	3.97	1.58	97.18
3	-do-	2.25	0.25	−0.05	30.93
4	-do-	2.02	0.41	−0.25	59.11
5	-do-	0.13	4.32	−1.43	96.00
6	-do-	3.35	1.29	−0.43	92.82
7	-do-	3.92	1.02	−0.60	93.77
9	-do-	4.70	1.08	−0.40	96.48
10	-do-	4.36	0.64	−0.23	88.57
11	-do-	3.75	1.05	−0.38	91.03
12	-do-	0.17	5.87	−2.22	90.91
13	-do-	1.94	3.98	−1.23	97.39
14	-do-	4.55	1.48	−0.48	86.71
15	-do-	0.84	5.66	−1.92	99.35
17	-do-	3.47	3.36	−1.21	95.60
18	-do-	3.65	1.58	−0.54	94.70
19	-do-	1.35	2.54	−0.92	85.31
20	-do-	3.57	1.52	−0.88	82.10

These changes have been quantified in terms of various equations for the individual soils. Based on the patterns obtained, the following generalizations may be made for predicting the changes.

Water soluble P (ppm):

$$Y = a + bt + ct^2$$

for soils low to medium in clay and medium to high in C content.

$$Y = a + b \ln (t + 1) + c [\ln (t + 1)]^2$$

for soils with high Fe and clay content.

$$\ln Y = a + b \ln (t + 1) + c\,[\ln (t + 1)]^2$$

for soils medium in clay and medium to high in C.
Available Fe and Mn (ppm):

$$\ln Y = a + b \ln (t + 1) + c\,[\ln (t + 1)]^2$$

where Y = parameter studied at "t" days of submergence, "a" is a constant and "b" and "c" are the regression coefficients.

*

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PROBLEMS OF ATHALIA ROSAE L. (HYM.: TENTHREDINIDAE) IN HUNGARY

Winter rape (*Brassica napus* L. f. *biennis* Thell.) is grown at present in Hungary on 50,000 hectares and the sowing area increases from year to year. Spring rape (*B. napus* L. f. *annua* Thell.) is not grown in Hungary. The average yield of winter rape varies between 10 and 16.5 quintals per hectare; among the factors influencing the yield *Athalia rosae* plays a prominent role. This fact is reflected also in earlier Hungarian literature (Horváth 1884, Jablonowski 1893) according to which *A. rosae* is the most important rape pest in Hungary.

By studying the literature of 70 years, reports on heavy damages caused by this pest show a certain periodicity of 5—8 years. In my regular observations, however, carried out for 20 years, I could not detect any regularity in the mass swarming of the pest. It is my opinion that the degree of infestation on rape depends on the connections of swarming phenology and plant phenology, i.e. the presence of susceptible plant stages. Namely, if the swarming of the second summer generation coincides with plant stages optimal for egglaying (plants with 4—5 leaves) one may expect a heavy infestation. If the rape plants are more advanced at this time, the damage will be only moderate.

A. rosae swarms three times a year in Hungary. The overwintering occurs in the eonymphal stage in the soil, inside a cocoon. The swarming of the overwintered generation begins in the first days of May and lasts about 35 days (until June 10). The peak of swarming occurs between May 20 and 30. This generation lays its eggs on the cultivated mustard (*Sinapis alba* L.) or on *S. arvensis* L. The first summer generation swarms between the end of June and July; the females of this generation lay their eggs mostly into pioneer plants, grown from seeds which had dropped from the pods at harvest. The second summer generation begins its swarming at the end of August and the swarming proceeds until the beginning of October (Sáringer 1957a, 1957b, 1961). From an economic standpoint, the progeny of this generation is the most dangerous, which is further stressed by the fact that this generation shows the highest number of individuals (Fig. 1).

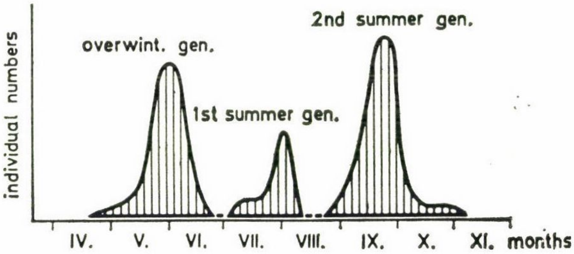

Fig. 1. Swarming of different generations of *Athalia rosae* in Keszthely (Southwest Hungary, 1960—1970)

The number of generations per year of *A. rosae* is regulated by the combined effect of photoperiod and temperature. In Fig. 2 the results of larval rearing experiments are shown, carried out at three constant temperatures and different photoperiods. According to these, the diapause occurring in the eonymphal stage at 18 and 22 °C is induced mainly by the photoperiod. At 28 °C and at even higher temperatures the effect of photoperiod is substantially modified by the temperature. The photophase critical for diapause falls at 18 and 22 °C between 14 and 15 hours (Sáringer 1964). It is the mature larva which is sensitive to the photoperiod, between the termination of feeding and entering into the soil for pupation, i.e. for only 3—4 days (Sáringer 1967). I did not find any connection between the serial number of the generation to which the individual belonged and the per cent of diapause, in experiments carried out at 22 °C and 17/7 photoperiod for 11 consecutive generations. The diapausing larvae can be reactivated in 2—3 weeks at high temperatures (28—32 °C). The geographical conditions of Hungary, situated between 46—48° latitude and 16—23° longitude provide favourable climatic (temperature, photoperiod) conditions for three swarmings a year. It was thus established that *A. rosae* is a long-day species with facultative diapause.

The larvae consume 86.5 per cent of their total larval food on rape, on mustard 88.9 per cent in the last two developmental stages (SÁRINGER 1957a). This fact stresses the importance of controlling this pest at the beginning of larval development.

From among the natural enemies *Tachina nigricans* Egger and *Perilampus aeneus* Rossi were reared; the rate of parasitization, however, always remained under 10 per cent.

The control of *A. rosae* is carried out on a farm scale in two ways (by two methods) worked out by the author:

1. Soil and crop management practices, by which the younger ($L_2 - L_4$) larvae of the second summer generation, developing on stubble-grown rape are ploughed under by the end of July. In one of our large agricultural farms no other method was used for 11 years; the otherwise necessary chemical treatment (in September) could be omitted. The stubble-grown rape acted as green manure and resulted in a yield increase of 80—120 kg/hectare in the wheat grown afterwards. This method needs consideration also from the point of view of environmental protection.

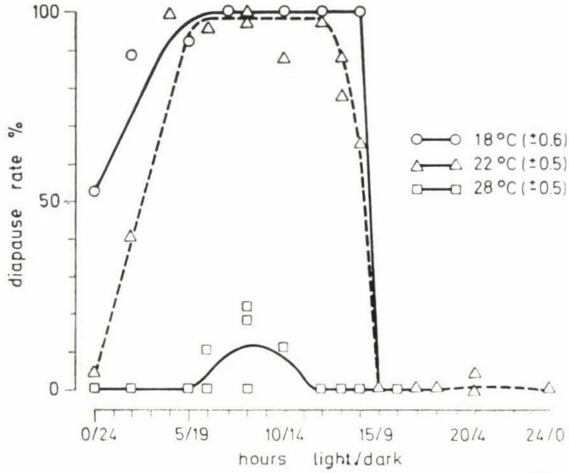

Fig. 2. Diapause curves of *Athalia rosae* at different temperatures and photoperiods (after Sáringer 1964)

2. In chemical treatments during September—October Wofatox Spritzpulver 30 and Methylparathion WP are sprayed (0.4 — 0.5%) or Wofatox dust is applied (17.5—20 kg/hectare) at the time of the young larval stages.

A new, ecological control method is being worked out. The method — still in the experimental stage — is based on the susceptibility of larvae against photoperiod. Larvae reared at a daylength typical for September (at a photoperiod shorter than the critical daylength) show two light sensitive periods in the scotophase. If in these periods the larvae receive a short (30 minutes) light exposure, they develop without diapause. A control method consists of a "photoflash" treatment; during the nights of September or October the larvae are illuminated from 11.30 p.m. to 12 p.m. and from 3 a.m. to 3.30 a.m. by using reflectors of a car or tractor. These short light impulses hinder the larval diapause, and the adults emerge in the autumn. The oviposition on the senescent rape leaves, however, is unsuccessful and this leads to a substantial decrease in population density.

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Prepared at the Field Laboratory of the Research Institute for Plant Protection, Keszthely, Hungary

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EFFECT OF PRE-HARVEST SPRAYS OF SOME GROWTH RETARDANTS ON THE QUALITY OF "BANATI" GRAPES DURING COLD STORAGE

The investigation was started with the hope to study the effect of some growth retardants on the fruit quality and to prolong the fruit life span in cold storage of some grape varieties in the United Arab Republic. This was achieved through the use of the growth retardants Alar and cycocel.

Alar treatment increased the weight of cherries (Ryuzo 1966), and hastened fruit colouration of apples (Edgerton *et al.* 1966). On the contrary, it was reported that Alar delayed the colouration of apple fruits Southwick *et al.* (1968), and had no effect on soluble solids in apples and cherries (Ryuzo 1966). On the other hand, Edgerton—Hoffman (1965) and Williams *et al.* (1964) reported that Alar treatments increased the soluble solids in apples, while Looney *et al.* (1967) on apples and Griggs—Iwakiri (1968) on pear mentioned that Alar reduced the soluble solids. Martin *et al.* (1968) showed that there was no significant effect of Alar on the titrable acids of apple fruits. In some cases Alar increased the acidity of apples (Williams *et al.* 1964), while reduced it in others (Looney *et al.* 1967).

This investigation was carried out on the Thompson seedless "Banati" grape variety (nineteen years old), planted at the Faculty Research Station, Cairo University. The selected vines were uniform and with good growth vigor. Three concentrations of both Alar and cycocel were used. The concentrations were 500, 1000, and 2500 p.p.m. As a result we had seven treatments including the control. Each treatment consisted of ten vines. Each vine was sprayed alone with five liters of solution which was enough for through tree drenching. In all treatments "Tipol" was added to the spraying solutions in a concentration of 0.001 as a spreader. The vines of every treatment were sprayed once just after fruit setting.

All vines were treated alike as far as agricultural practices were concerned (manuring, irrigation, pest control, etc.). A completely randomized block design was used. The work was carried out for two successive seasons. The methods recommended by El-Azzouni—El-Mahdi (1960) for picking grape bunches were applied in this investigation.

Mature "Banati" grape bunches were picked in the morning and then transported immediately to the laboratory. Samples of about 50 bunches from every treatment were washed with tap water, dipped in a 4 per cent borax solution for 3 minutes at a temperature of 32 °C, rinsed with tap water, then spread on tables and subjected to electric fans for about one hour to get dry. Finally grape bunches were packed in carton boxes (60 × 40 × 15 cm) in one layer and stored at 0 °C. At 2 weeks intervals, samples were taken for both physical and chemical analysis.

It is evident from Table 1, that the juice percentage of all the treated and untreated fruits showed a trend of a gradual decrease with the advance of the storage period. At the end of the storage period, nearly all the treated fruits showed a higher juice percentage than the untreated fruits (control) excluding some slight fluctuations. The fruit juice percentage from vines sprayed with 1000 p.p.m. of either Alar or cycocel solutions showed higher juice per cent followed by those sprayed with either Alar or cycocel at concentrations of 2500 p.p.m. and 500 p.p.m. respectively. The results of the two seasons are similar. Statistically the differences between the treated and the untreated fruit, within the treatments, and the periods of storage are significant. The results agree with those found by Ryuzo (1966) on cherries.

It is obvious from Table 2 that the colour of the treated and the untreated fruits advanced with the progressing of the storage period. The rate of colour advancement was quicker in the control than in the treated fruits. This result agrees with those of Southwick *et al.* (1968), and disagrees with those of Edgerton *et al.* (1966).

Table 3 illustrates that the flavour of the treated and the untreated fruits showed an improvement with the advance of the storage period. In the fruit of vines sprayed with either 1000 or 2500 p.p.m. Alar or cycocel the excellent flavour appeared earlier (after 10 weeks from storage) than in those sprayed with 500 p.p.m. (after 12 weeks from storage), while the untreated fruits showed the excellent flavour more earlier (after 8 weeks from storage). The two seasons showed similar results.

Table 4 illustrates the presence of a gradual increase in the total soluble solids of both the treated and untreated fruits, reaching their maximum values at the end of the storage period. There are no significant differences between the treated and the untreated fruits and within the treatments from a statistical point of view. The only significant differences are found between the periods of storage. This result agrees with those of Ryuzo (1966) and disagrees with those of Williams *et al.* (1964).

It is obvious from Table 5, that the total acidity of both the treated and untreated fruits showed a gradual and continuous trend of decrease with the advancement of the storage period until reaching their minimum values at the end of storage period. Fruits sprayed with 1000 p.p.m. from either Alar or cycocel showed the lower values of acidity at the end of the storage period, followed by those sprayed with 2500 p.p.m. and then those sprayed with 500 p.p.m. from either Alar or cycocel, excluding some fluctuations. At the end of the storage period, the control showed higher values of total acidity. Similar results were found between that of the two seasons. Statistically the results were significant within treatments, the treated and the untreated fruits and between the periods of storage. This result agrees with those of Looney *et al.* (1967).

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Table 1

The effect of spraying the vines with different concentrations of Alar and cycocel on the juice

Periods of storage (weeks)	Season I									
	Alar				cycocel				cont.	Total
	500	1000	2500	Sum	500	1000	2500	Sum		
2	71.4	73.3	71.1	215.8	69.3	71.9	70.6	211.8	70.2	497.8
4	70.0	70.8	67.5	208.3	68.6	70.6	70.4	209.6	67.9	485.8
6	70.6	71.0	64.7	206.3	67.8	66.7	69.7	204.2	68.0	478.5
8	63.2	64.5	65.0	192.7	64.5	64.1	66.7	195.3	63.7	451.7
10	63.2	63.2	62.1	188.5	61.4	64.3	63.7	189.4	63.9	441.8
12	60.0	62.5	61.3	183.8	57.7	62.8	66.8	182.3	59.9	426.0
Total	398.4	405.3	391.7	1195.4	389.3	400.4	402.9	1192.6	393.6	2781.6

Table 2

The effect of spraying vines with different concentrations of Alar and cycocel on the colour of "Banati" grapes, cold stored at 0 °C

Periods of storage (weeks)	Season I							Season II						
	Alar			Cycocel			cont.	Alar			Cycocel			cont.
	500	1000	2500	500	1000	2500		500	1000	2500	500	1000	2500	
2	1	1	1	1	2	1	2	1	2	1	1	2	1	2
4	2	2	2	3	3	3	4	2	4	3	2	4	2	3
6	3	3	4	4	4	4	5	3	5	4	3	4	4	5
8	4	5	5	5	5	5	5	4	5	5	5	5	4	5
10	5	6	5	5	6	6	6	4	6	5	5	6	5	6
12	6	6	6	6	6	6	6	6	6	6	6	6	6	6

Colour		Symbol
Agathia green	60/2	1
Pea green	61/1	2
Pea green	61/2	3
Sap green	62/2	4
Pea green	61/3	5
Uranium green	63/2	6

According to the Horticultural Colour Chart
Issued by the British Colour Council

percentage of "Banati" grapes, cold stored at 0 °C

Season II										
Alar				cycocel				cont.	Total	Grand Total
500	1000	2500	Sum	500	1000	2500	Sum			
70.2	72.9	70.8	213.9	70.1	72.0	70.0	212.1	70.2	496.2	994.0
69.6	71.7	68.0	209.3	69.4	71.0	69.7	210.1	65.5	484.9	970.7
63.8	70.5	65.4	199.7	67.5	70.8	68.8	207.1	66.0	472.8	951.3
62.7	68.3	66.0	197.0	63.7	66.1	64.4	194.2	63.4	454.6	906.3
63.0	62.3	60.5	185.8	62.0	64.6	60.9	187.5	62.2	435.5	877.3
60.2	61.9	60.4	182.5	60.0	63.0	61.2	184.2	60.1	426.8	852.8
389.5	407.6	391.1	1188.2	392.7	407.5	395.0	1195.2	387.4	2770.8	5552.4

L.S.D. for treatments ± 0.05 = 1.1.
L.S.D. for treatments VS. control ± 0.05 = 1.50
L.S.D. for periods of storage ± 0.05 = 1.2.

Table 3

The effect of spraying the vines with different concentrations of Alar and cycocel on the flavour of "Banati" grapes, cold stored at 0 °C

Periods of storage (weeks)	Season I							Season II						
	Alar			Cycocel			cont.	Alar			Cycocel			cont.
	500	1000	2500	500	1000	2500		500	1000	2500	500	1000	2500	
2	1	1	1	1	1	1	2	1	2	1	1	2	1	1
4	2	2	2	2	3	2	3	2	3	3	2	3	2	3
6	2	4	3	3	4	3	4	3	4	3	3	4	4	4
8	3	4	4	4	4	4	5	4	4	4	4	4	4	5
10	4	5	5	4	5	5	5	4	5	4	4	5	5	5
12	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Flavour	Symbol
Sweet	1
Sweet, fair flavour, slight aromatic	2
Sweet, fair flavour, and aromatic	3
Sweet, good flavour, and aromatic	4
Sweet, excellent flavour, and aromatic	5

Table 4

The effect of spraying the vines with different concentrations of Alar and cycocel on

Periods of storage (weeks)	Season I									
	Alar				cycocel				cont.	Total
	500	1000	2500	Sum	500	1000	2500	Sum		
2	19.5	19.5	17.5	56.5	17.0	19.5	17.5	54.0	18.0	128.5
4	19.5	20.0	18.0	57.5	18.5	20.0	18.0	56.5	19.5	133.5
6	21.5	21.0	19.0	61.5	19.5	20.0	20.0	59.5	19.5	140.5
8	21.0	22.0	19.5	62.5	20.0	20.5	20.5	61.0	20.0	143.5
10	21.5	22.0	20.0	63.5	20.0	20.5	20.0	60.5	20.0	144.0
12	22.0	23.0	21.0	66.0	21.0	22.5	21.0	64.5	21.0	151.5
Total	125.0	127.5	115.0	367.5	116.0	123.0	117.0	356.0	118.0	841.5

Table 5

The effect of spraying the vines with different concentrations of Alar and cycocel on the

Periods of storage (weeks)	Season I									
	Alar				cycocel				cont.	Total
	500	1000	2500	Sum	500	1000	2500	Sum		
2	1.02	0.95	1.49	3.46	1.10	0.98	1.02	3.10	1.11	7.67
4	0.98	0.90	1.16	3.04	1.07	0.97	0.98	3.02	1.01	7.07
6	0.90	0.92	1.03	2.85	0.96	0.90	0.90	2.76	0.95	6.56
8	0.90	0.88	0.97	2.75	0.98	0.84	0.92	2.74	0.89	6.38
10	0.87	0.84	0.95	2.66	0.79	0.80	0.87	2.46	0.83	5.95
12	0.78	0.64	0.89	2.31	0.77	0.75	0.72	2.24	0.83	5.38
Total	5.45	5.13	6.49	17.07	5.67	5.24	5.41	16.32	5.62	39.01

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the total soluble solids of "Banati" grapes, cold stored at 0 °C

Season II										
Alar				cycocel				cont.	Total	Grand Total
500	1000	2500	Sum	500	1000	2500	Sum			
19.0	19.5	18.5	57.0	18.0	19.5	19.0	56.5	18.0	131.5	260.0
19.5	20.0	19.0	58.5	19.5	19.5	19.5	58.5	18.5	135.5	269.0
20.5	21.0	20.0	61.5	20.5	20.0	19.5	60.0	19.0	140.5	281.0
20.5	21.5	20.0	62.0	20.0	20.5	20.5	61.0	20.5	143.5	287.0
21.5	22.0	21.0	64.5	21.0	21.5	20.0	62.5	20.0	147.0	291.0
21.5	22.5	21.0	65.0	21.5	23.0	21.5	66.0	21.0	152.0	303.5
122.5	126.5	119.5	368.5	120.5	124.0	120.0	364.5	117.0	850.0	1691.5

total acidity of "Banati" grapes, cold stored at 0 °C

Season II										Grand Total
Alar				cycocel				cont.	Total	
500	1000	2500	Sum	500	1000	2500	Sum			
1.19	1.01	1.21	3.41	1.20	1.00	1.19	3.39	1.26	8.06	15.73
1.03	0.94	1.10	3.07	1.14	0.92	1.11	3.17	1.17	7.41	14.48
0.94	0.90	1.00	2.84	0.95	0.93	0.99	2.87	1.03	6.74	13.30
0.89	0.92	0.92	2.73	0.96	0.80	0.91	2.67	0.90	6.30	12.68
0.85	0.82	0.88	2.55	0.90	0.84	0.88	2.62	0.85	6.02	11.97
0.86	0.74	0.81	2.41	0.81	0.76	0.80	2.37	0.87	5.65	11.03
5.76	5.33	5.92	17.01	5.96	5.25	5.88	17.09	6.08	40.18	79.19

L.S.D. for treatments ± 0.05 = 0.05.
L.S.D. for treatments VS. control ± 0.05 = 0.07.
L.S.D. periods of storage ± 0.05 = 0.04.

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RYE INFECTION EXPERIMENTS WITH LYOPHILIZED CLAVICEPS PURPUREA SPORES

With the view of obtaining ergot, this important pharmaceutical basic material in large quantities and an adequate uniform quality the artificial infection of rye plants with *Claviceps purpurea* spores has become necessary. A number of researchers, e.g. HECKE (1921, 1922) and FALCK (1922), tried to spray the spores into the open flowers of rye.

Infection independent of the flowering process of rye was solved by BÉKÉSY (1934) who introduced the spores into the rye ears by injection. This way of infecting with an injection syringe gave the idea of constructing mass-inoculation machines (BÉKÉSI 1938a, b, HECHT 1940), which after different modifications he mounted to tractors.

The improvement of the large-scale rye infection made it necessary to produce a larger quantity of *Claviceps purpurea* spores. Culturing of the *Claviceps purpurea* on artificial culture media has been attempted by many since TULASNE (1833) described the fungus in detail. In Hungary ROMÁN (1950) cultured the *Claviceps purpurea* spores required for the large-scale production of ergot first in Petri dishes, then later in "R" kolle tubes on the surface of an agar-agar culture medium of 8—10 per cent sugar content recommended by BÉKÉSY (1955). DIM-ZAJEC—MASTNAK (1951) found the cultures not older than four weeks the most suitable for performing inoculations. According to SOÓS (1969) the 12 weeks old cultures also gave good results.

The cultures have to be prepared 10—12 weeks before the inoculation, because aged spores are no longer suitable for infection. In order to obtain a larger quantity and more uniform infecting material GLÁZ (1955) propagated the *Claviceps purpurea* conidia in a fermentor. According to his investigations the *Claviceps purpurea* spores propagated in fermentors but washed off the surface of agar lost viability at +25 °C within two or three weeks. It is therefore a difficult task to preserve conidia propagated in great masses, and deliver them to the place of infection. According to his investigations the *Claviceps purpurea* spores did not stand drying up, but the conidia cooled to −33, −40 °C retained their germinative ability for 6 months. In consequence of all this our attention turned toward lyophilization, a technique widely used in the case of other microorganisms.

As to the lyophilization of *Claviceps purpurea* spores no reference has been found in the literature. The main task of our experiments was to preserve the conidia of *Claviceps purpurea* propagated in shaken cultures and fermentors by freezing drying, then with the thus preserved spores carry out rye infection experiments.

The experiments were carried out at the Research Institute for Medicinal Plants with the OY 178 ergotamine strain isolated by Békésy and used for large-scale ergot production in the same year. The cultures were produced by inoculating sterile pieces of the inner pseudo-parenchyma tissue of the sclerotium onto agar-agar culture medium. The material of the test-tube culture was propagated in "T" kolle tubes on malt-agar culture medium. Spores required for lyophilization were propagated on culture media recommended by GLÁZ (1955), in a way described by him. After the eighth or ninth day the cultures were filtered, then the filtrate centrifuged. A part of the spores was diluted with sterilized skimmed milk, the other part with horse blood serum; the latter contained 4 per cent saccharose too.

Lyophilization was carried out with the cryochem or vapour freezing method.* We used 1 ml 5—600,000/mm^3 spores per ampoule.

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* Cryochem method: only in the second phase, since the first phase of the Edwards 30 PS lyophilizator used by us is of a vapour freezing system (24 + 4 hour shift). Our vapour freezing system is Flosdorf—Mudd's glass equipment (4 hour shift).

Table 1

Rye infection experiments with lyophilized inocula
(Taminos strain)
(1961—1967)

Site, number and year of experiment	Treatments	Yield kg/ha	%	Total alkaloid content, %
Budakalász I.	1. Lyophilized (1961 serum)	217	115	0.36
1961	2. Lyophilized (1961 milk)	103	55	0.34
	3. Control (normal agar culture)	188	100	0.35
Albertirsa II.	1. Lyophilized (1961 serum)	163	75	0.36
1962	2. Lyophilized (1962 serum)	102	47	0.36
	3. Control (normal agar culture)	216	100	0.33
Budakalász III.	1. Lyophilized material (1962 serum)	138	87	0.34
1963	2. Lyophilized material (1963 milk)	170	108	0.34
	3. Control (normal agar culture)	158	100	0.33
Cinkota IV.	1. Lyophilized material (1962 serum)	85	109	0.33
1963	2. Lyophilized material (1963 milk)	88	113	0.37
	3. Control (normal agar culture)	78	100	0.35
Rákoskeresztúr V.	1. Lyophilized material (1961 serum)	107	81	0.35
1964	2. Lyophilized material (1963 milk)	105	79	0.34
	3. Control (normal agar culture)	132	100	0.34
Rákoskeresztúr VI.	1. Lyophilized material (1962 serum)	123	76	0.35
1965	2. Lyophilized material (1963 milk)	158	95	0.33
	3. Control (normal agar culture)	166	100	0.34
Rákoskeresztúr VII.	1. Lyophilized material (1962 serum)	69	49	0.35
	2. Lyophilized material (1963 milk)	79	56	0.36
	3. Control (normal agar culture)	140	100	0.35
Average of the seven	1. Lyophilized (serum)	128.8	83.6	—
experiments	2. Lyophilized (milk)	115.0	74.7	—
	3. Control (normal agar culture)	154.0	100.0	—
	S. D. 5%	38.6	25.1	

Both before and after the lyophilization the quantity of spores was determined in a Bürker chamber. The germinative ability was measured by their germination in a hanging drop preparation at +25 °C.

Before the use the ampoules were opened and a suspension of 20 × 10^3/mm^3 spores was made of them with physiological salt solution. This was used for infection. The small plot field experiments were laid out at Budakalász (Treatment I), Albertirsa (Treatment II), Budakalász (Treatment III), Cinkota (Treatment IV), Rákoskeresztúr (Treatment V), Rákoskeresztúr (Treatment VI), Rákoskeresztúr (Treatment VII). The experiments were carried out on small plots of 6 × 6 = 36 m^2 each, in the middle of an about 10 ha rye field after SARKADI (1962) in a Latin square design, with four replications.

On the proposal of BÉKÉSY (1956) the infection was made when the tip of the rye ear emerged from the leaf sheath. Treatment I was started on 17th May 1961, Treatment II on 12th May 1962, Treatment III on 22nd May 1963, Treatment IV on 3rd May 1963, Treatment V on 7–8th May 1964, Treatment VI on 10th May 1965, Treatment VII on 4th May 1967. Inoculation was carried out with hand plates described by BÉKÉSY (1947). The ergot produced on the field plots was collected with hand collecting devices.

As a result of our lyophilizing methods the number of spores was reduced by 10–20 per cent, and the germinative ability of spores decreased by 20–45 per cent. In a lyophilized state the spores could be stored for 3 years at 4 °C.

As a control, the yield attained with spores washed off the agar surface was taken for 100 per cent. Our lyophilized preparations were found to produce an essentially lower ergot yield than the infections made with suspensions washed off the agar surface. The treatment with the protective milk colloid showed a depressive effect significant at S.D. 5% on the average of the seven treatments. Between the serum-protective colloid and milk-protective colloid treatments significant difference could not be found. Further, it can be established that lyophilization did not change the total alkaloid content of the ergot.

ACKNOWLEDGEMENTS

We are indebted to Mr. M. Békésy (Research Institute for Medicinal Plants, Budapest) for placing the strains at our disposal as well as imparting his rich experiences and carrying out the total alkaloid examinations; to Mr. G. Szakmári (Phylaxia Veterinary Biologicals and Feedstuffs Co., Budapest) for preparing the lyophilized material.

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REGENERATION OF THE RICE PLANT FROM ROOT DERIVED CALLUS TISSUE

Induction of callus formation. The hulled rice seeds (*Oryza sativa* L. variety TB-2) were sterilized by soaking in 70 per cent ethanol and in 0.1 per cent $HgCl_2$ for appropriate time intervals. After washing carefully with sterilized water, the seeds were germinated in test tubes containing 10 ml of Murashige and Skoog revised medium (MURASHIGE—SKOOG 1962) at 30 °C in the dark.

After 4 weeks, intense callus growth was observed in the place of the seminal roots (Fig. 1). Friable callus tissues were then subcultured on the same medium. 2.4 D (2 mg/l) was found essential for callus induction. Increasing the concentration of 2.4 D up to 8 mg/l had no effect. Casein hydrolysate (1000 mg/l) and glycine (2 mg/l) did not improve the growth of the callus tissues.

Induction of root and shoot formation. Undifferentiated callus tissues (0.5—0.6 cm in diameter) were transferred to test tubes containing 2.4 D — free Murashige and Skoog medium to which B-indolacetic acid (2 mg/l) and kinetin (2 mg/l) were added. The test tubes were then kept at 30 °C under fluorescent light. After 2 weeks, small roots began to appear and grew intensively. 1—2 weeks later small groups of green cells differentiated from the upper surface of the callus. These cells were then developed into normal rice seedlings (Figs 2 and 3). The seedlings were first transferred to a low osmotic pressure medium (saccharose cencentration was reduced to 1 per cent) and then to sterilized sand. The pots were watered with Kasugai mineral solution. After a "lag" period of some weeks, the young seedlings grew normally and showed development of ears and ripening of grains. No phenotypes of peculiar appearance were observed although Nishi et al. (NISHI—YAMADA—TAKAHASHI 1968) reported that about 20 per cent of the restored rice plants had chromosomal aberration.

The restoration of the rice plant from root derived callus tissue on a well defined medium offers an ideal system to study the role of growth regulators in the organ differentiation of higher plants.

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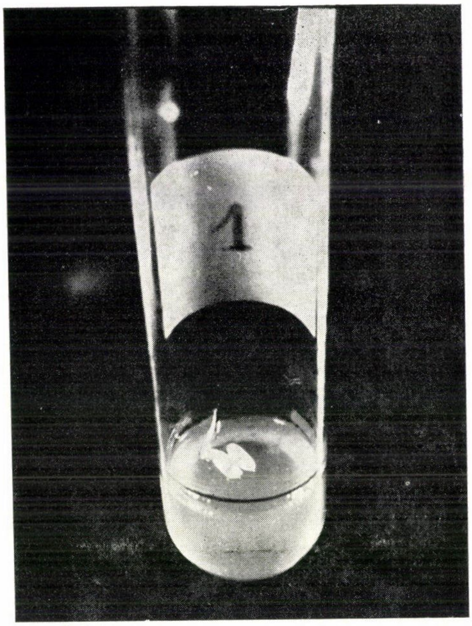

Fig. 1. Callus formation from rice seminal root

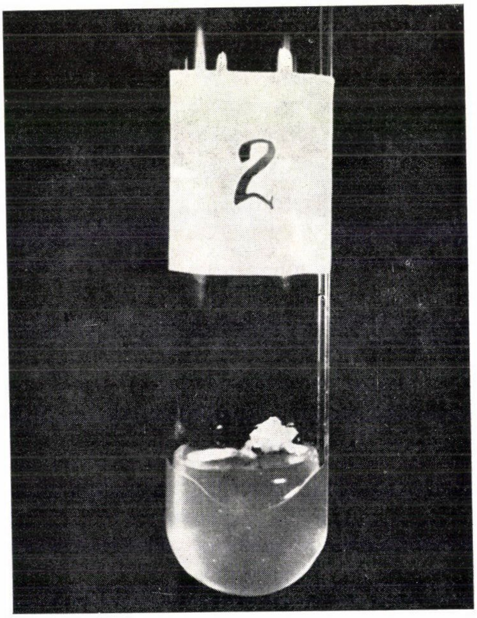

Fig. 2. Root derived callus after subculture

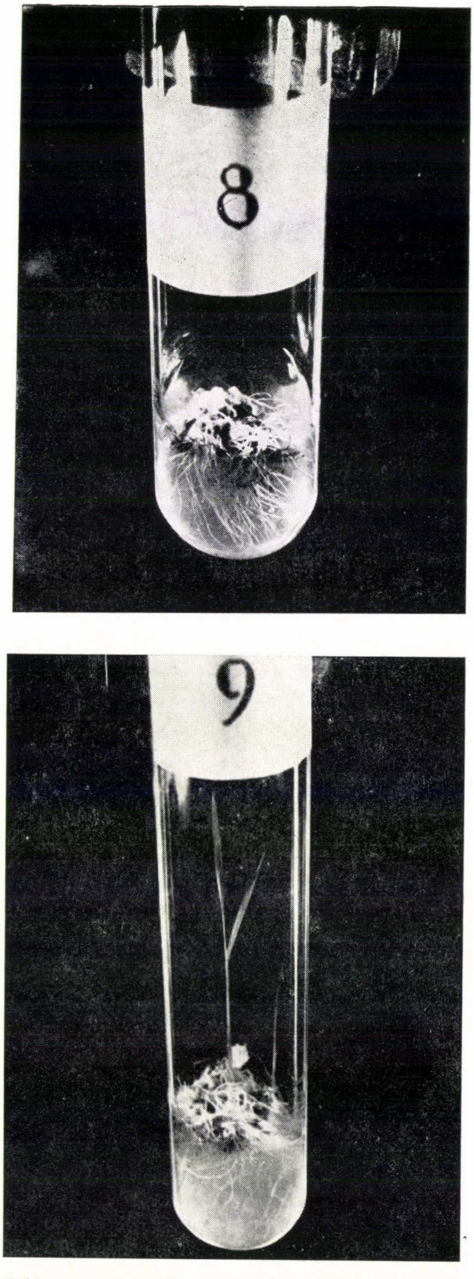

Fig. 3. Redifferentiation of roots (A) and shoots (B) from callus

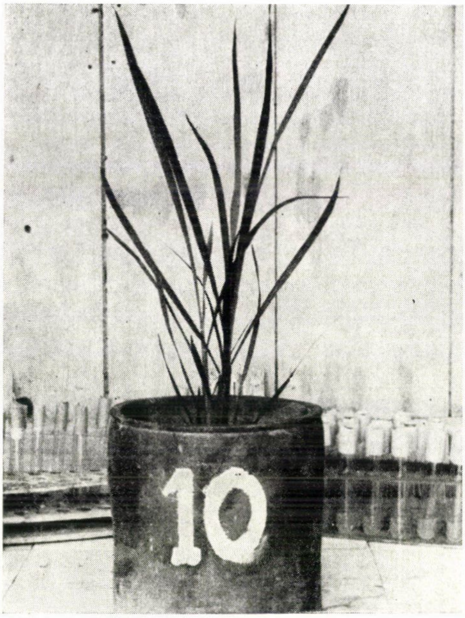

Fig. 4. The whole rice plant redifferentiated from callus grown on field soil

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TREATMENTS INCREASING FRUIT SETTING IN PÁNDY SOUR CHERRY CLONES. I. POLLINATION WITH POLLEN MIXTURES

Literary data on the enhancing effect on the fruit set of pollination with pollen mixtures are not unambiguous for the sour cherry varieties. According to Enikeev (1947) in the case of a pollen mixture the extent of fruit setting was higher than when pollination was carried out with the pollen of a single variety. Kolesnikov (1959) and Cociv—Gozob (1960—1961) obtained a poorer fruit setting with pollen mixtures than when using the pollen of the varieties represented in the mixture separately. In the case of Pándy sour cherry Nagy (1965) found that the pollen mixture did not give a better fruit setting than the best pollen variety represented in the mixture.

One of the most important problems of sour cherry production is to ensure a regular, not fluctuating, increase in the yield. In Hungary the Pándy sour cherry and various types of Cigány sour cherry planted as pollen varieties have been grown for decades.

The Pándy sour cherry and its types are totally self-sterile. Its macrospore is deficiently fertilized, it produces little pollen, is stuck together and is highly sterile. Late flowering is also an obstacle to cross-pollination in the sour cherry Pándy (Nyéki 1974).

Three types of treatment for increasing the extent of fruit setting were examined: pollination with pollen mixtures, supplementary pollination (pollen blowing) and repeated pollination. In this publication only the results of pollination with pollen mixtures are discussed. The fruit setting studies were carried out in 1972 and 1974 at the Érd-Elvira Station of the Horticultural Research Institute with a collection of sour cherry varieties on Prunus mahaleb rootstock planted in 1955. In the course of the investigations the flower buds were isolated with waterproof parchment bags. In each treatment 5—9 isolators were used. The calculations were made on the basis of the proportion of set (ripe) fruits to the total number of treated flowers. 10—57 flowers per isolator were pollinated. Each of them is regarded as a replication. Pollen fertility was examined with true-bred pollen and mixtures of 1 : 1 ratio

Table 1

Fruit setting in Pándy sour cherry-279 clone with a pollen mixture of Germersdorfi óriás-3 clone and Solymári gömbölyű cherry varieties
(1972, Érd—Elvira)

Pollination ♂	Number of pollinated stigmae	Fruit setting (%)
with the pollen of Germersdorfi óriás-3 clone	26	3.8
	34	11.8
	20	15.0
	26	18.5
	31	19.4
variety average	164	14.0
with the pollen of Solymári gömbölyű	27	0.0
	50	2.0
	48	4.2
	32	15.6
	36	27.8
variety average	193	9.3
with a 1 : 1 ratio pollen mixture of Germersdorfi óriás-3 clone + Solymári gömbölyű	27	3.7
	20	10.0
	30	13.3
	57	17.5
	30	30.0
pollen mixture average	164	15.9
Control (Pándy-279 clone, free pollination)	1001	2.0

Table 2

Fruit setting in Kőrösi sour cherry (Pándy-26 clone) when pollinated with a pollen mixture of Germersdorfi óriás-57 clone and Solymári gömbölyű cherry varieties
(1974, Érd—Elvira)

Pollination ♂	Number of pollinated stigmae	Fruit setting (%)
with the pollen of Germersdorfi óriás-57 clone	14	0.0
	18	0.0
	21	0.0
	24	0.0
	20	0.0
	25	4.0
	19	5.3
	17	6.0
	16	6.3
variety average	174	2.4
with the pollen of Solymári gömbölyű	22	0.0
	21	0.0
	18	0.0
	22	0.0
	21	0.0
	20	5.0
	10	10.0
	14	14.0
variety average	148	3.6
with a 1 : 1 ratio pollen mixture of Germersdorfi óriás-57 clone + Solymári gömbölyű	27	0.0
	23	0.0
	24	0.0
	15	0.0
	20	0.0
	16	6.3
	19	11.0
	19	11.0
	20	15.0
pollen mixture average	183	4.8
Control (Kőrösi sour cherry, free pollination)	690	4.8

In the course of the experiment we studied whether

- pollination with pollen mixtures increased the extent of fruit setting in the sour cherry as in other fruit species;
- pollination with a pollen mixture increased the extent of fruit setting compared to the best pollen variety represented in the mixture;

- in the individual treatments there is any difference in the extent of fruit setting when true-bred or mixed pollen is used.

Fruit setting in Pándy sour cherry-279 clone with a pollen mixture of Germersdorf óriás and Solymári gömbölyű cherry varieties is shown in Table 1, and in Kőrösi sour cherry (Pándy sour cherry-26 clone) in Table 2.

Fruit were set in 14.0 per cent of the flowers of Pándy-279 clone when pollinated with the pollen of Germersdorfi óriás-3 clone, and in 9.3 per cent when pollinated with the variety Solymári gömbölyű. The pollen mixture of Germersdorfi óriás-3 clone and the variety Solymári gömbölyű produced a 15.9 per cent fruit setting. The free-pollinated control set fruit in 2.0 per cent. Compared to the best pollen variety represented in the mixture pollination with a pollen mixture gave a 1.9 per cent higher fruit setting. The pollen mixture was 6.6 per cent more effective than the pollen of the variety Solymári gömbölyű.

In 1972 the dispersion per replication was lower in Germersdorfi and higher in Solymári gömbölyű. In contrast to this, while the extent of dispersion did not change, the average fruit setting percentage slightly increased with the pollen mixtures. Besides, pollination with a pollen mixture is more reliable than that with true-bred pollen. A similar — though less marked — tendency was observed in 1974, which from the point of view of fruit setting was a critical year owing to low temperatures at the time of flowering.

The flowers of Kőrösi sour cherry (Pándy-26 clone) were fertilized in 2.4 per cent by the pollen of Germersdorfi óriás-57 clone, and in 3.6 per cent by pollen from Solymári gömbölyű. When pollinated with a pollen mixture the Kőrösi sour cherry set fruit in 4.8 per cent. Similar fruit setting was shown by the free-pollinated control flowers too.

Compared to the best pollen variety represented in the pollen mixture (Solymári gömbölyű) fruit setting was 1.2 per cent better when pollination was carried out with a pollen mixture. Compared to the fertilizing ability of the Germersdorfi óriás-57 clone, pollination with pollen mixtures resulted in a 2.4 per cent higher fruit setting.

On the basis of the experimental data we can establish the fact that under favourable conditions pollination with pollen mixtures increased the extent of fruit setting compared to the control, while under conditions unfavourable for fruit setting the results did not differ from those obtained with the control.

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STIGMA RECEPTIVITY IN MALE STERILE WHEAT

Stigma receptivity and time of flowering of male sterile and restorer lines are important factors in determining the extent of outcrossing which is a pre-requisite for using heterosis in wheat. The possibility of the commercial production of hybrid wheat in many respects depends upon the solution of this problem. A characterization of this attribute will, therefore, be extremely helpful in evaluating the degree of outcrossing in relation to hybrid seed production under given environmental conditions. In view of the above fact and also inspired by the studies made by IMRIE (1966), RAJKI—RAJKI (1966) and ABRAMOVA (1966) the present problem was undertaken to study the receptivity of stigmae of different male sterile lines involving *Triticum timopheevi* and *Aegilops caudata* cytoplasms under varying weather conditions.

Four male sterile lines — three from *T. timopheevi* source (m.s. 4696, m.s. Sonora-64 and m.s. Caprock) and one from *Ae. caudata* source were selected for the study. Eleven to fifteen ears of the same age, that had just emerged out of the boot, were covered with crossing bags after removing all but eight spikelets in each head and two flowers in each spikelet, the total being 16 flowers. From the first day of flowering upto the 15th day, each day one head of each female line was hand pollinated. An attempt was made to provide adequate pollen to all the heads pollinated on different days. Other factors were kept constant. Percentage of seed set per head over the different days of pollination gave an estimate of stigma receptivity and was calculated as

$$\frac{\text{Number of seeds set}}{\text{16 (total number of ovules)}} \times 100$$

Relative humidity and temperature at the time of pollination and seed set were also recorded.

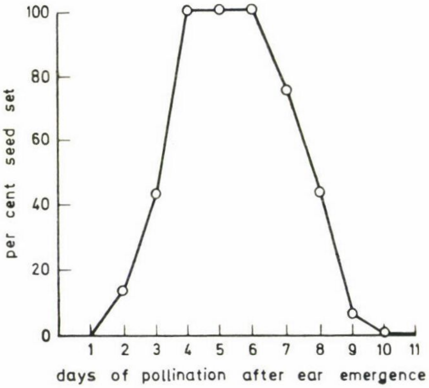

Fig. 1. Stigma receptivity in M. S. Sonora-64

The seed set in the selected heads of the four male sterile lines indicated the stigma receptivity. The results have been summarized in Table 1a, b, c, d. All the four female lines showed a similar trend of stigma receptivity, indicating thereby no influence of cytoplasm on stigma receptivity. The effect of weather conditions, temperature and relative humidity was conspicuous. During the month of February pollination after one day of ear emergence did not produce any seed. Highest stigma receptivity was observed on the 4th, 5th and 6th day after ear emergence. From the 7th day upto the 9th day it decreased rapidly and stigmae

Table 1

Stigma receptivity in four male sterile lines of wheat
(a) m.s. 70B, 11—4696 *T. timopheevi*
(Feb. 2 to 12)

Dated: February	2	3	4	5	6	7	8	9	10	11	12
No. of seeds set	0.0	1.0	5.0	16.0	16.0	15.0	16.0	10.0	3.0	0.0	0.0
% of seed set	0.0	6.3	31.3	100.0	100.0	93.8	100.0	62.5	18.75	0.0	0.0
Temp. Max.	25.5	19.7	20.3	21.4	17.5	20.0	19.4	20.7	22.0	23.5	21.5
Min.	13.4	11.3	6.3	8.1	5.9	5.3	4.1	3.3	4.1	5.9	7.6
Relative humidity	93.0	93.0	92.0	93.0	94.0	83.0	75.0	69.0	85.0	89.0	88.0
				Cloudy	Cloudy						

(b) m.s. Sonora-64 *T. timopheevi*
(Feb. 7 to 17)

Dated: February	7	8	9	10	11	12	13	14	15	16	17
No. of seds set	0.0	2.0	7.0	16.0	16.0	16.0	12.0	7.0	1.0	0.0	0.0
% of seed set	0.0	12.5	43.8	100.0	100.0	100.0	75.0	48.8	6.25	0.0	0.0
Temp. Max.	20.0	19.4	20.7	22.0	23.5	21.5	23.7	18.0	19.8	21.4	22.6
Min.	5.3	4.1	3.3	4.1	5.9	7.6	6.8	4.3	4.1	4.1	6.6
Relative humidity	83.0	75.0	69.0	85.0	89.0	88.0	78.0	88.0	84.0	75.0	72.0

Table 1 *(cont.)*

(c) m.s. Salmon *Ae. caudata*
(March 3 to 15)

Dated: March		3	4	5	6	7	8	9	10	11	12	13	14	15
No. of seeds set		1.0	6.0	11.0	16.0	15.0	15.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0
% of seed set		6.3	37.5	68.8	100.0	93.8	93.8	12.5	0.0	0.0	0.0	0.0	0.0	0.0
Temp.	Max.	27.7	29.0	21.6	24.0	24.0	24.2	26.3	29.9	29.0	30.5	26.5	31.0	29.2
	Min.	10.3	12.6	11.8	11.4	11.8	13.1	12.9	11.4	11.1	16.4	16.7	13.1	12.8
Relative humidity		47.0	54.0	83.0	93.0	82.0	84.0	65.0	81.0	78.0	81.0	64.0	43.0	59.0

(d) m.s. Caprock *T. timopheevi*
(March 4 to 18)

Dated: March		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
No. of seeds set		3.0	9.0	16.0	15.0	16.0	16.0	5.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% of seed set		18.8	56.3	100.0	93.8	100.0	100.0	31.3	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Temp.	Max.	29.0	21.6	24.0	24.0	24.2	26.3	29.2	29.0	30.5	26.5	31.0	29.2	30.0	28.4	32.0
	Min.	12.6	11.8	11.4	11.8	13.1	12.9	11.4	11.1	16.4	16.7	13.1	12.8	14.6	15.9	14.4
Relative humidity		54.0	83.0	93.0	82.0	84.0	65.0	81.0	73.0	81.0	64.0	43.0	59.0	58.0	67.0	56.0

ceased to be receptive from the 10th day onward. Figure 1 shows the trend of stigma receptivity in m.s. Sonora-62 which represents four other male sterile lines also.

A similar experiment was conducted in the month of March when the temperature had increased considerably. The stigma was found to be receptive even after one day of ear emergence. The highest receptivity was on the 3rd, 4th, 5th and 6th day and it ceased to be receptive after eight days of ear emergence. This early receptivity may be attributed to high temperature and low relative humidity. This confirms the observations of IMRIE (1966) who found that relative humidity and hot drying winds influenced the period of stigma receptivity. Similar observations were reported by other workers as BRADIER (1960) and RAJKI (1961) who also recorded a strong influence of weather conditions on stigma receptivity. These observations are further supported by DEVRIES (1971) who, by free pollination under moderate conditions, obtained the highest percentage of seed set on the third and fourth day after start of flowering while on the seventh day there was practically no seed set.

According to PORTER *et al.* (1967) proper nicking of the male sterile and restorer line is a primary factor determining seed set on the male sterile line. Based on the observations in the present study it may be foreseen that a restorer producing abundant pollen on the 3rd, 4th, 5th and 6th day after ear emergence with properly nicking male sterile lines will ensure increased percentage of seed set on male sterile lines. This will result in higher hybrid seed produced per unit area and make the hybrid seed production more economical.

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DIFFERENTIAL TOXICITY OF CADMIUM IN RICE VARIETIES

Cadmium should be considered in the array of metal pollutants due to its possible chronic effects (SORSA—PFEIFER 1973). This requires continuous monitoring and frequent investigations. In the human body there is a clear positive correlation between the cadmium content and age as well as the degree of industrialization of the environment (NILSSON 1970).

Japan being a highly industrialized country, reports on severe effect of cadmium pollution are sometimes found in the newspapers and the Japanese Government recently put an alarm on such pollutants.

Cadmium is found in living organisms mostly due to its use as metallic coatings against corrosion, in preparing metal alloys and as a pigment for painting. Cadmium yellow is renowned for its permanent yellow and water insoluble nature. It is found as an impurity in zinc products (SORSA—PFEIFER 1973). Cadmium was found to be a constituent of automobile tires, gasoline and oil. It has also been found in soil and grass samples along road-ways (LAGERWERFF—SPECHT 1970, MILLER *et al.* 1973).

The waste-products from various industries in Japan are drained out in sewage canals or through streams without proper care. These streams or sewage canals meet the water requirements of rice fields in Japan. The unfathomable danger this may inflict on mankind is often reflected by the swollen outgrowths in fishes and sometimes by death. This leads to a probable danger that cadmium might be incorporated in the human body through food (rice).

Table 1

List of varieties used

Name of variety	Identification number	Place of origin	Type
Chuh-tu	109	Taiwan	Indica
Nagae-wase	538	Japan	Japonica
Padi-ase-banda (Gotta-bog)	647	Indonesia (Celebis-Island)	Japonica
Liu-tou-tu	143	Taiwan	Indica
R. T. S. 23	60	Vietnam	Indica
Chin-tsao	727	South China	Indica
O-hou-tsao-chiu	734	South China	Japonica

The present investigation deals with the effect of cadmium concentrations on rice, with a hope to find varietal differences in cadmium tolerance capacities.

From a large collection of rice varieties seven varieties (Table 1) were selected at random and seeds of these varieties subjected to five different treatments including one untreated (control) lot for each variety. The cadmium was used in the form of a solution of $CdSO_4$ in distilled water. Four replications containing one hundred seeds for each treatment of each variety were imbibed in cadmium solutions of 0, 1, 5, 25 and 125-ppm concentrations and were kept soaked in the solutions respectively until data were taken. The experiment was conducted in a phytotron (4000 lux of light, 25 ± 1 °C, 60—70% of R. H.), to grow the seedlings under uniform environmental conditions. It was assumed that with the help of uniform environmental conditions, a true representation of the varieties would be obtained with a small number of plants. During the growing period no mineral nutritions were added to avoid interactions. The germinability was noted within the first ten days and seedling heights within twenty days. The seedling heights were expressed in the ratio of the control seedling heights, to avoid varietal differences.

Regression analysis was done to find the differential cadmium tolerance in rice varieties. In these calculations the basic mean performances were measured on a logarithmic scale, since it was observed that by this means a high degree of linearity was induced in the regressions. Use of a logarithmic scale also induced a reasonable degree of homogeneity of experimental error. It should be noted that mean performances on a logarithmic scale correspond to geometric means on the natural scale, instead of the more usual arithmetic means.

The mean performance of all varieties for each treatment allowed a quantitative grading of the treatments based on their effects on the character concerned and have been represented as the abscissa. The ordinate in the same graph represents the individual variety (mean). From the slope of the regression the nature of the cadmium tolerance of the variety has been described, following Finlay—Wilkinson (1963). The more the regression line is horizontal, the greater tolerance to cadmium it represents. Contrarily, as the regression line becomes verticaland approaches the regression value (b) of 1.0, it represents an average cadmium tolerance. A regression value (b) of more than 1.0 indicates a below average cadmium tolerance, or a sensitivity to cadmium.

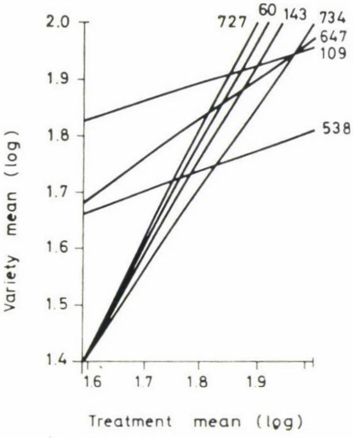

Fig. 1. Regression lines for germination, showing individual performances under differen treatment conditions

High levels of cadmium retarded the seedling height and germination severely. In almost all varieties at the highest concentration only two leaves were produced. The leaves were very thin and yellowish in colour. The margins of the leaves were somewhat whitish and the uppermost part of the leaves remained unfolded. The roots developed a brownish colour, that could easily be removed by scraping with a knife. The resistant varieties showed deeply green leaves at a lower concentration. Though the chlorotic nature of the leaves and the brownish pigment in the roots does not show a step-wise increment towards higher concentrations, the 125-ppm showed the maximum.

Table 2 shows the effect of cadmium on germination and seedling height. It may be observed from the columns of Table 2 that according to varieties and concentrations of cadmium the germination and seedling height varied considerably.

Table 3 and Fig. 1 for germination shows that different degrees of cadmium tolerance exist among varieties. The varieties 538, 109 and 647 were found to be comparatively resistant; 538 being the most resistant.

Similarly, for seedling height, the regression values showed that only the variety 109 is sufficiently below 1.0 (Table 3) and the slope of the regression line (Fig. 2) is more horizontal than those of the others, indicating more tolerance than in other varieties. The results thus

Table 2

Effect of cadmium concentrations on germination and seedling height

Variety	Seeds		Mean seedling height (in cm)	Ratio of seedling height (% of control)
	sown	germinated		
109/control	100	98	2.86	
109/1 ppm	100	100	2.43	84.99
109/5 ppm	100	93	3.24	113.42
109/25 ppm	100	80	0.93	32.39
109/125 ppm	100	65	0.83	28.89
538/control	100	86	5.82	
538/1 ppm	100	80	3.93	67.53
538/5 ppm	100	72	3.84	65.97
538/25 ppm	100	52	1.57	27.01
538/125 ppm	100	44	0.11	1.87
647/control	100	100	9.44	56.73
647/1 ppm	100	96	5.36	56.73
647/5 ppm	100	84	4.71	49.93
647/25 ppm	100	82	3.71	39.31
647/125 ppm	100	50	0.11	1.17
143/control	100	100	4.92	
143/1 ppm	100	96	4.83	98.25
143/5 ppm	100	92	4.79	97.57
143/25 ppm	100	88	1.66	33.83
143/125 ppm	100	26	0.59	12.13
60/control	100	100	10.53	
60/1 ppm	100	98	5.06	48.07
60/5 ppm	100	100	4.97	47.16
60/25 ppm	100	100	2.23	21.15
60/125 ppm	100	000	0.00	0.00
727/control	100	100	12.19	
727/1 ppm	100	100	3.76	30.82
727/5 ppm	100	100	3.99	32.79
727/25 ppm	100	96	2.33	19.14
727/125 ppm	100	12	0.98	7.99
734/control	100	100	7.89	
734/1 ppm	100	100	4.42	56.03
734/5 ppm	100	100	4.36	55.26
734/25 ppm	100	68	2.61	33.15
734/125 ppm	100	000	0.00	0.00

obtained clearly show that rice varieties differ in cadmium tolerance capacities, and germination and seedling height are not always interdependent with regard to cadmium tolerance.

Regarding the cadmium action mechanism, Miller *et al.* (1973) have shown in an experiment with corn mitochondria that cadmium stimulated the oxidation of NADH and the presence of phosphate negated it. But in the presence of phosphate, succinate or malate + pyruvate it was oxidized more than when phosphate was absent. They suggested that the site of the cadmium effect is likely to be early in the electron transport and the cytochrome b complex is the probable site. They also mentioned that "on a percentage basis, the inhibition of substrate oxidation was, regardless of the substrate, present at lower Cd^{+2} concentrations than were the inhibitions of coupling as determined by ADP/O and respiratory control ratios (RCR). Such data could be used to hypothesize a site of Cd^{+2} effect on the phosphorylative mechanism which is unique from the effect on electron transport presented earlier."

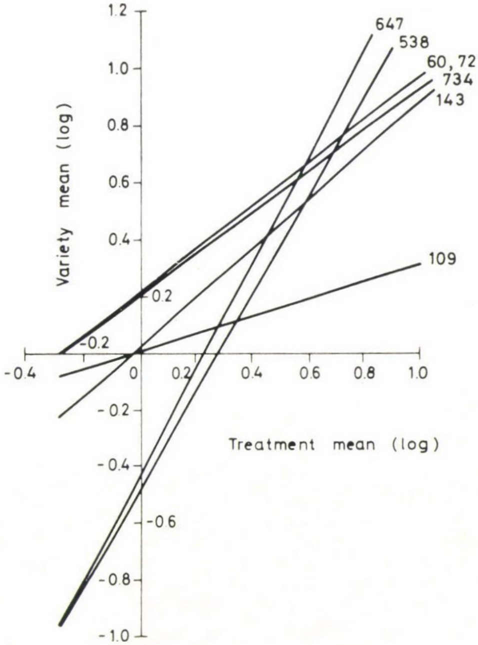

Fig. 2. Regression lines for seedling height, showing individual performances under different treatment conditions

Nilsson (1970) reported that ionic cadmium reacts with sulfh ydryl groups, its cytogenetic mode of action most probably is connected with — either directly of indirectly — the formation and function of the mitotic apparatus. Evidence of this type of mutagenicity was obtained in plant material (Nilsson 1970). Miller *et al.* (1973), however, showed that Dithiothreitol, a SH group protector, prevented any effect of cadmium, which suggests that sulfhydryl groups are likely to be involved in the action mechanism.

As far as the present investigation is concerned, it may be suggested that deep-green leaves at a lower concentration and very thin yellowish leaves at a higher concentration are indicators of polyploidisation at a lower concentration and termination of growth and cell death at a higher concentration. This implies that cadmium might have interrupted the spindle formation at the divisional stage and ultimately caused cell death, which are in line with Nilsson (1970).

Table 3

Regression coefficients of germination and seedling height

Varieties	Germination	Seedling height
109	0.5647	0.3377
538	0.6316	0.7322
647	0.7638	1.2246
143	1.2245	0.6668
60	1.7287	1.4673
727	1.5329	1.5367
734	1.7537	1.1265
734	1.7537	1.1265

ACKNOWLEDGEMENT

Thanks are due to Prof. D. Moriwaki, Director, National Institute of Genetics, Misima Japan, for facilities to carry out this work.

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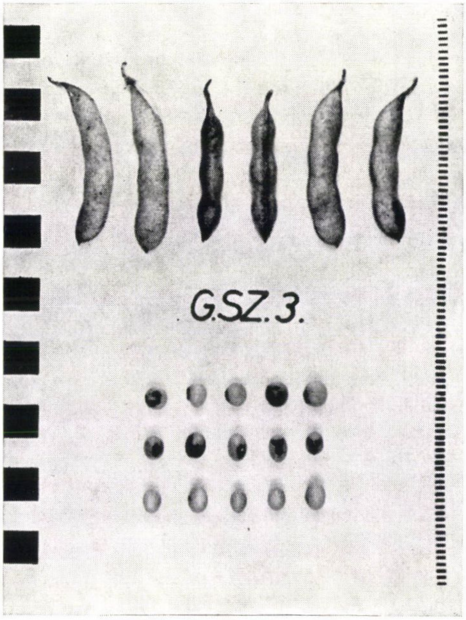

G. SZ. 3 SOYBEAN

Taxonomical place: *Glycine soya* (L.) Sieb. et Zucc. ssp. *manshurica* Enk.
Origin: Aprószemű Sárga × Universal
Beginning of breeding: 1960, Iregszemcse
Breeders: Ernő Kurnik and Anna Oberritter, Iregszemcse
State qualification: provisionally certified improved variety 1968, state certified variety 1974.
General characterization: due to its vegetative character it yields a good green fodder; it is tolerant to drought, does not require irrigation but ripens late.

Morhological description:

Root system: its main root system is richly branching, with a strong main root. Many *Rhizobium*-tubers grow on the root system (Szabó in litt.).

Shoot system: 75—100 cm high, fairly branching, with pale yellow (sometimes brownish) hairs on the surface.

Stem: dark green (the hypocotyle is at first violescent and bare), hairy, with a medium tendency to lodging (stem stability is 2.8 (5 is the best)).

Foliage: dark green, with ovate, pointed leaflets covered by yellowish-brown hair.

Inflorescence: cluster with few flowers in the axil of leaf.

Flowers: tiny, with violet corolla.

Fruit: slightly bent or straight, sword-like legume with 2—3 seeds in it; when ripe the fruit is covered by pale brown, short hair.

Seed: round, medium large (6—7 mm in diameter), of bright yellow basic colour mottled with dark brown or black. The hilum is black, its narrow ring dark brown. Thousand-seed-weight is 138—179; 165 g on an average. The crude protein content of seed is 37 (—40) per cent, the raw fat content 17—18 per cent (Bakos 1971).

Biological characters:

Germination: under favourable conditions its germinative ability is 93—95 per cent, but may even fall to 80 per cent under unfavourable conditions.

Vegetative period: from shooting to flowering 51 days, the total period of vegetation 130—149 days (late ripening).

Water requirement: drought tolerant; due to its low stability irrigation is not recommended (OMFI 1969), but it may still give a seed yield of 29 q/ha (BAKOS 1971).

Resistance to disease: it shows a medium susceptibility to green virus (OMFI 1969).

Farm technology requirements:

Sowing: largest yield is obtained by sowing 530 000—700 000 germs per ha (OMFI 1969).

Soil requirement: in soils rich in nutrient it yields well.

Productivity: seed yield 19.8—20.3 q/ha (or more when irrigated). The hay contains 17—18 per cent crude protein (OMFI 1969).

Region of cultivation: it can be grown everywhere in Hungary, but most efficiently in the southern region.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 183—208 (1976)

FORUM

INDIRECT EXAMINATION OF THE ROLE OF FORMALDEHYDE AND GLYCOLALDEHYDE IN CARBON METABOLISM

By the establishment of the CALVIN (1955) cycle a pathway of photosynthesis that is found in photosynthetizing unicellular as well as higher plants and become known. The biochemical pathway of photosynthesis in green plants together with the photosynthetic form found by KORTSCHAK *et al.* (1965) and HATCH—SLACK (1966) to dominate in C_4 and CAM plants has become in principle so well known that trying to fing any other way seems to be superfluous. Some literary data suggest, however, the possible existence of a third way of carbon dioxide fixation, though its role is limited compared to the mentioned two processes.

BALDRY *et al.* (1966) reported that the ability of spinach chloroplast to photosynthetize $C^{14}O_2$ was considerably reduced when glycolaldehyde was added to it. According to O'NEAL *et al.* (1972) glycolaldehyde or glycolaldehyde phosphate added to chloroplast prepared from a 4—5 days old maize plant resulted in a 30—90 per cent inhibition of $C^{14}O_2$ photosynthesis. According to the most plausible explanation of this phenomenon it is the continued building up of the non-radiating glycolaldehyde rather than the fixation of the radiating carbon dioxide that takes place in this case and causes the reduction of carbon dioxide fixation. GIBBS (1966) and BAMBERGER—GIBBS (1965) were the first to study the effect of non-radiating carbohydrates on the fixation of radiating carbon dioxide. This started an interesting series of experiments. SCHACTER *et al.* (1971) studied the effects of intermediates in the process from carbon dioxide fixation to carbohydrate formation in the presence of inhibitors too. They found that the inhibiting effect of iodoacetic acid and arsenite could be eliminated by introducing fructose-1.6-diphosphate, or glyceraldehyde-3-phosphate and ribose-5-phosphate, respectively. Moreover, not only the carbon dioxide fixation but the amount of the released oxygen too was restored even though Ru-5-P kinase is inhibited by both the iodoacetic acid and arsenite, and the glyceraldehyde-3-P dehydrogenase by the iodoacetic acid (CALO—GIBBS 1960, GIBBS—CALO 1960). This means that the two inhibitors hinder the functioning of the Calvin cycle. The two inhibited processes form an important part of carbon dioxide fixation in C_4 plants too, since the fixed carbon dioxide in the bundle sheath cells is built up to carbohydrates by means of the two reactions. The only explanation for the inhibition-releasing effect of the mentioned three intermediates is that with their help a photosynthetic pathway in which the active presence of Ru-5-P kinase and glyceraldehyde-3-P dehydrogenase is not needed becomes dominant. The results obtained so far suggest that in a possible third photosynthetic process glyceraldehyde-3-P, Fr-1.6-DiP and Ri-5-P play a role similar to that of the carbon dioxide acceptor. On the basis of observations made by BALDRY *et al.* (1966) and O'NEAL *et al.* (1972) it is possible that the carbon dioxide is somehow first built up to glycolaldehyde and takes part in the further processes in that form.

The third photosynthetic pathway is made highly probable by the early appearance of glycolic acid. ZELITCH (1959) pointed out that 50 per cent of the radiating carbon of photosynthetically fixed carbon dioxide was found in the glycolic acid inhibiting the glycolic acid oxidase, and the two carbon atoms of the glycolic acid showed the same intensity of radiation which suggests that both carbon atoms were produced from freshly assimilated carbon dioxide. It was again ZELITCH (1965) who noticed that in tobacco leaves the specific activity of glycolic acid produced during the photosynthesis of the radiating carbon dioxide exceeded the specific activity of PGA. Although it is true that a considerable number of data point to the formation of glycolic acid during photorespiration too, this does not exclude the possibility of photosynthetic formation. The diversity of literary data makes it probable that glycolic acid can be formed both by photosynthesis — from freshly assimilated carbon dioxide — and by photorespiration. It is easy to suppose that if glycolaldehyde is formed in the course of photosynthesis, it may be oxidized into glycolic acid. This process must be brought into connection with the participation of the transketolase enzyme. According to WILSON—CALVIN (1955) with the participation of transketolase glycolic acid may be formed from pentose phosphate. DATTA—RACKER (1961) pointed out that with transketolase a glycolaldehyde-transketolase complex was formed from Fr-6-P. BRADBEER—RACKER (1961) gave evidence of the thus produced glycolaldehyde being oxidized to glycolic acid even by ferricyanide. SHAIN—GIBBS (1971) pointed out the light dependent formation from Fr-6-P of a considerable amount of glycolic acid under the influence of transketolase enzyme added to spinach chloroplast. It is thus a proved fact that with the participation of transketolase glycolic acid can be formed from glycolaldehyde. A large proportion of the publications tried to prove that glycolic acid (and glycolaldehyde, its precursor) were only formed by photorespiration, from carbon dioxide, and not by photosynthesis. The fact published by SCHACTER *et al.* (1971) that the introduction of Fr-1.6-DiP and Ri-5-P reduces the quantity of glycolic acid formed during photosynthesis confirms the idea that the photosynthetically fixed carbon dioxide can be built up through glycolaldehyde. The further building up of the thus formed glycolaldehyde is promoted by Fr-1.6-DiP, Ri-5-P and glyceraldehyde-3-P. If the building up of the formed glycolaldehyde is inhibited owing to the low concentration of these compounds, then the glycolaldehyde is changed to glycolic acid. The fact, however, that glycolic acid is formed in this case too, and that this compound is produced by photorespiration suggests that the glycolic acid has some kind of physiological role too.

On the basis of the above, the formation of glycolaldehyde after the reduction of the two molecules of carbon dioxide, as well as its further building up can be imagined. It remains to be found out what one-carbon-atom compound can be derived from the carbon dioxide from which glycolaldehyde can be produced too. Carbon dioxide reduced to formaldehyde and built up to glycolaldehyde was one of the possible solutions, although in this case the line of research does not agree with Bayer's conception, since the basic idea was born from the results of investigations carried out with chloroplast suspensions prepared from plants.

Our objective was to find out whether formaldehyde and glycolaldehyde can play the role of intermediary in the photosynthetic building up of carbon dioxide to carbohydrates, and if so what light reaction conditions are required for the process. Since formaldehyde has not been pointed out in plants so far — partly on account of analytical difficulties, partly because of its presumably very low physiological concentration —, we had to choose an indirect method of analysis. We added the assumed intermediates to the subject of analysis and measured the quantities of carbohydrates formed from the intermediates under the influence of light. The conclusions were drawn in an unusual way. We concluded on the presence of an already known process, or in the case of more that one simultaneous processes on the dominance of inferiority of one of the processes from the changes observed in the quantity of the measured monosaccharides.

In the first phase of the investigation we tried to decide whether the living plant was able to form carbohydrate from formaldehyde, that is, whether the plant could take up gaseous formaldehyde instead of carbon dioxide and form carbohydrates from it in a similar way.

On the other hand, we wanted to find out whether by the change of the concentration of formaldehyde harmful physiological processes were caused, and what the concentration at which the harmful processes would not occur was.

In the preliminary experiment three lucerne (alfalfa) plants of nearly identical weight deprived of their roots were placed in Knop solution. One of the plants was left in the air of the laboratory as a control. The second plant was hermetically sealed by a bell jar, and saturated KOH solution and soda lime were placed under the bell jar to reduce the carbon dioxide content. The third sample was placed under the same conditions with the exception that in the airspace a 0.5 mg% formaldehyde concentration was established. The plants were kept over 72 hours under these conditions. In an experiment of this type changes in the dry matter content could not be unambiguously followed, since the nearly identical weights of the fresh samples did not always imply identical dry matter contents (the ratio of leaf to stem was not the same), and the standard deviation was, accordingly, great in the replications. However, the sulfhydryl content of the fresh sample, the soluble and total nitrogen content of the dried sample, the amino acid content of the hydrolysed samples (paper chromatography) and the soluble and total carbohydrate quantities could be measured well (Nosticzius 1966b). Substantial differences were found in the carbohydrate content as shown in Table 1.

Table 1

Quantitative changes of carbohydrates in dry matter percentage under the influence of a relatively small quantity — 0.5 mg% — of formaldehyde present in the air
(Nosticzius 1966a)

Treatment	Reducing		Total		Percentage increase			
					reducing		total	
	water soluble	hydrolysed	water soluble	hydrolysed	water soluble	hydrolysed	water soluble	hydrolysed
Untreated	0.833	4.930	2.995	5.762	0.00	1.39	15.19	0.44
reduced CO_2	0.833	4.862	2.600	5.737	0.00	0.00	0.00	0.00
formaldehyde	1.533	5.894	3.182	6.475	84.03	21.22	22.38	12.86

At the same time, no actually measured difference was found in the soluble and total amount of nitrogen as a response to the formaldehyde content of the air-space (Table 2).

The dried samples were hydrolysed and their amino acid contents studied by paper chromatography. On the basis of the size of the spots no difference was found in the amino acid content between the samples of the different treatments.

Further on we examined in which part of the plant the formaldehyde caused the most essential change; with this in view rooted lucerne plants grown in pots were treated under glass bells in the same way as before, but leaves, stems and roots were examined separately. The results are given in Table 3.

The most significant change was observed in the root. Changes occurring in the dry matter content were followed in an indirect way. 12 g of each of three rootless lucerne plants was placed in 80 ml Knop solution and treated in the above described way over 72 hours. After this the carbohydrate content was determined in the Knop solution. This amount of carbohydrate can be regarded as a quantity sent by the plant towards the root, that is, as an increase in the dry matter content. The results are shown in Table 4.

Table 2

Nitrogen contents in plants grown in bell jar for 72 hours

Treatment	Soluble	Total	Percentage of soluble to total nitrogen
	N/100 g dry matter		
Reduced CO_2	1.42	3.49	40.69
Formaldehyde (0.5 mg%)	1.41	3.47	40.63

Table 3

Quantitative changes of carbohydrates in different plant parts as expressed in dry matter percentage
(Nosticzius 1966a)

Treatment	Reducing		Total		Percentage increase			
					reducing		total	
	water soluble	hydrolysed	water soluble	hydrolysed	water soluble	hydrolysed	water soluble	hydrolysed
Reduced CO_2								
leaf	0.099	0.225	1.68	3.32				
stem	0.124	1.812	1.96	6.16				
root	2.905	5.930	1.845	12.85				
Formaldehyde (0.5 mg%)								
leaf	0.124	0.575	1.81	4.53	25.25	55.55	7.73	36.44
stem	0.121	1.600	1.76	5.64	−3.42	−11.70	−10.21	−8.45
root	3.701	8.450	3.00	20.15	27.40	42.49	62.60	56.81

Table 4

Carbohydrates moving to the roots in green plants
(Nosticzius 1966a)

Treatment	Total carbohydrates in 10^{-4} g glucose	Percentage of hexose to total carbohydrates	Percentage increase of total carbohydrates
Untreated	5.408	80.11	14.67
reduced CO_2	4.716	74.62	0.00
formaldehyde (0.5 mg%)	13.212	90.40	180.15

The question arises whether the formaldehyde causes this type of change at any concentration, or its increasing concentrations start harmful physiological processes. When we increased the concentration of formaldehyde to 5 mg%, the amount of carbohydrates no longer grew in the dry matter content. Table 5 shows the quantities of carbohydrates in the roots of lucerne plants treated in the above described way, but at a formaldehyde concentration increased tenfold. In the leaves and roots of the plants the amount of carbohydrates practically did not change; on the basis of previous observations the greatest change was expected to occur in the root, and here the relatively large quantity of formaldehyde in the air even caused a slight decrease.

At the same time a considerable change occurred in the compounds containing nitrogen. The results of nitrogen content measuring are shown in Table 6.

Table 5

Quantitative changes of carbohydrates in lucerne roots under the influence of a relatively large quantity of formaldehyde

Treatment	Percentage of total hydrolysed carbohydrates to dry matter	Percentage decrease compared to the control
Untreated	10.76	
reduced CO_2	9.44	12.27
formaldehyde (5 mg%)	9.21	14.40

Table 6

Changes in the nitrogen content under the influence of a relatively large amount of formaldehyde
(Nosticzius 1966b)

Treatment	Total	Soluble	Percentage of soluble to total nitrogen
	g N/100 g dry matter		
Untreated	4.753	1.882	39.62
reduced CO_2	4.665	1.750	37.51
formaldehyde (5 mg%)	4.321	1.145	26.50

The most remarkable change found by the paper chromatographic examination of amino acids in the hydrolysed samples was the intensive decrease in the amount of tyrosine and appearance of the spot of a new amino acid identified as cysteic acid in the sample treated with formaldehyde. We could only imagine the formation of cysteic acid from tyrosine through the replacement of the ringed part of tyrosine by the —SH radical further oxidized to a sulpho group. In green plants it was easy to point out the decrease in the amount of sulfhydryl radicals under the influence of formaldehyde (Nosticzius 1966b).

From the above we can draw the conclusion that the living plant is able to build up formaldehyde to carbohydrate; this compound may thus be intermediate in a photosynthetic process. However, its physiological concentration in the plant must be low, because when increased by an order of magnitude it induces harmful processes, and carbohydrate formation does not occur.

Further on we worked with plant homogenates and chloroplast suspensions. Respiration in the dark could be influenced by the mitochondria content, while photorespiration by choosing the proper plant. We tried to find a way of influencing the light induced building up. The light induced carbohydrate change was measured by adding the supposed intermediates of photosynthetic carbohydrate formation according to the number of carbon atoms — formaldehyde, glycolaldehyde and glyceraldehyde, respectively — to the chloroplast suspension or plant homogenate. In a part of the experiments spectrophotometry was only used for measuring the changes in the total amount of carbohydrates and in the amount of hexoses and pentoses (Nosticzius 1967). In another part of the experiments changes occurring in the quantity of the individual monosaccharides were also measured. The light induced growth of carbohydrates occurring in the presence of intermediates was regarded as the photosynthetic activity of the chloroplast suspension on the intermediate. With the decrease in the amount of carbohydrates occurring in the dark in samples not treated with intermediates glycolysis and dark respiration, while with the light induced reduction of carbohydrates photorespiration were characterized. So an opportunity arose to follow the processes of glycolysis and dark respiration, photorespiration, building up in the dark and photosynthetic building up merely on the basis of changes in the amount of carbohydrates. The method of ion exchange column chromatography is suitable for determining the quantitative changes of the seven most frequent monosaccharides, but it requires tremendous work and time. Therefore we had to determine the optimum time of incubation at which all four processes could be measured without disturbing each other's activities. To make the evaluation reliable solutions treated in different ways were simultaneously used. The "blank" solution represented the amount of carbohydrates originally present in the chloroplast suspension. In that case the amount of carbohydrates in the chloroplast was measured without incubation. The only difference between the so called untreated solutions and the "blank" solution was that one of the former was incubated in the dark and the decrease in its carbohydrate content relative to that in the "blank" solution was considered as dark respiration. The carbohydrate reduction in the untreated solution incubated in light relative to the one incubated in the dark was attributed to photorespiration. Samples containing intermediates were also incubated both in dark and in light. The carbohy-

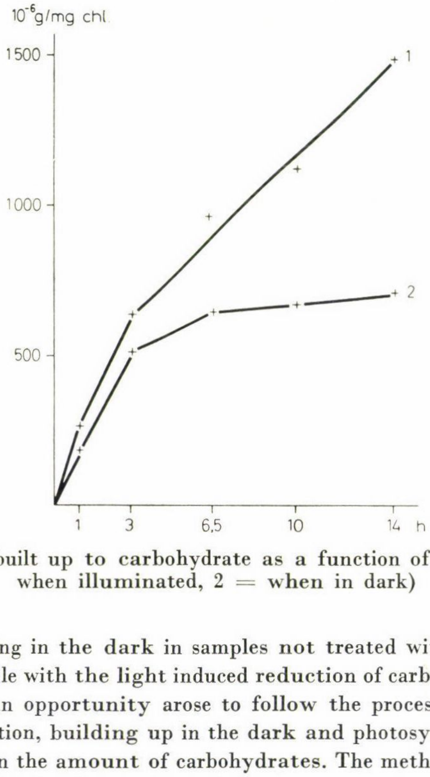

Fig. 1. Glyceraldehyde built up to carbohydrate as a function of the induction time (1 = when illuminated, 2 = when in dark)

drate increase in the intermediate-treated sample incubated in the dark compared to the untreated sample incubated in the dark was regarded as a building up process occurring even in the dark. When subtracting the carbohydrates of the untreated sample incubated in light from the values of the intermediate-treated sample incubated in light we obtained the sum of dark and photosynthetic building up. By this type of evaluation reliable results should be obtained even with a relatively long incubation time.

Short incubation periods are not suitable to make the effect of light felt, because the applied three intermediates are in a state already reduced to the carbohydrate level, therefore building up taking place even in the dark is relatively high. Fig. 1 shows the growth of carbohydrates in a chloroplast suspension treated with glyceraldehyde. Curve 1 represents the carbohydrate growth of the illuminated samples, while curve 2 shows the increase of carbohydrates in samples incubated in the dark. As seen from the figure only values obtained with a relatively long incubation time are suitable to give information about the light induced process of building up, as the difference between the effects of dark and light will only be significant in this case.

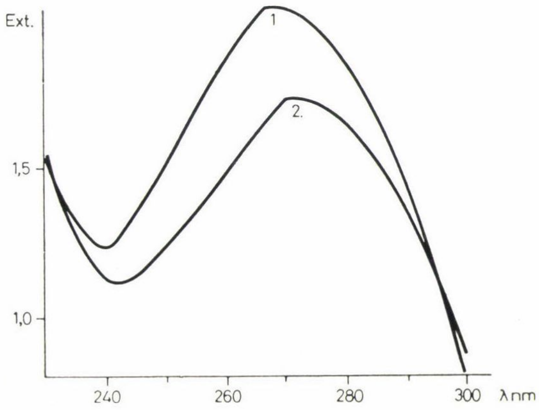

Fig. 2. Light absorption of samples treated with glyceraldehyde (1 = when illuminated, 2 = when in dark)

It seemed probable, however, that the intermediates had an influence not only on the carbohydrate formation but on the respiration processes too. To be able to evaluate the individual processes (glycolysis and dark respiration, building up in the dark, photosynthetic building up) we have to make the examined process dominant and reduce the three others to minimum. We had to find, therefore, the possibility of controlling the photosynthetic building up from the three examined intermediates. On the basis of the literature on light reaction it was expected that this form of building up also required the two light reactions, through the alternation of which an influence on the intensity of the photosynthetic building up could be attained.

Andersen *et al.* (1972) reported that the photosystem 2 (PS 2) was lower in the bundle sheath fraction of maize than in the mesophyll fraction. According to Smillie *et al.* (1972) by means of the PS 2 the bundle sheath fraction is able to carry out the NADP photoreduction too if plastocyanin is added to the system, but at the same time this increases the value of the photosystem 1 (PS 1) as well. Polya—Osmond (1972) point out that in C_4 plants the PS 2 value is much lower in the bundle sheath fraction than in the mesophyll fraction. The statement of Park—Sane (1971) that the stroma lamellae practically ensures the PS 1 capacity only, while the grana lamellae possesses both the PS 1 and PS 2 capacities well agrees with the data of the former authors, since the bundle sheath cells only contain a very low number of grana lamellae.

The above described suggest that the Hatch—Schlack—Kortschak process requires the PS 2 to a much higher degree than the Calvin cycle. So it seemed probable that the supposed gradual carbon number dependent photosynthetic building up of carbohydrates also required PS 2 and PS 1 in a definite ratio.

It occurred to us, however, that during the photosynthetic building up of intermediates not only carbohydrates but also compounds of other nature may have been formed, which influenced the evaluation of the data of photosynthetic actiityv based exclusively on the quantitative measuring of carbohydrates. The data of Table 2 as well as the chromatographic amino acid analysis performed at that time made it unlikely that essential quantities of amino acid and protein should be produced from the intermediates. There remained, however, the possibility that basic components of nucleic acids be formed. We did not intend to measure the components one by one and therefore studied the possibility of obtaining a reference to the formation of nucleic bases by using spectrophotometry as the only method. The limpid solution obtained after the incubation was photometried in the ultraviolet spectral range without any reagent added to it. It was a question whether the thus obtained extinction values really indicated the quantitative changes of the nucleic bases. The most outstanding increase of extinction was obtained in the case of chloroplast suspensions treated with glyceraldehyde. In Fig. 2 the values of the glyceraldehydetreated sample incubated in light are shown by curve 1 and those of the sample incubated in dark by curve 2.

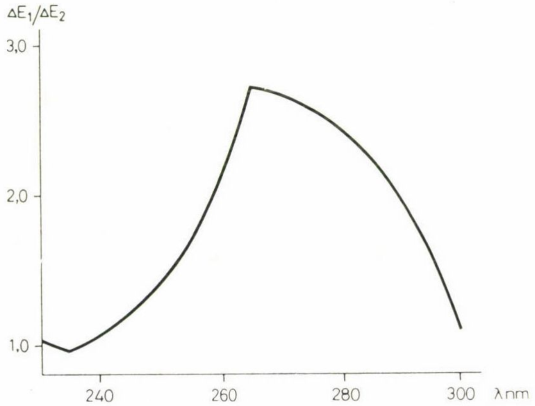

Fig. 3. Hyperchromic effect

To represent the fact that an important role is played here by the basic components of the nucleic acids too (besides AMP, ADP and ATP) we used the hyperchromic effect. Owing to the disintegration of H-bridge bonds the light absorption of nucleic acids increases on heating, and decreases again when they are cooled down.

We can get a very clear picture by taking the light absorption values of the intermediate-treated light-incubated sample before and after placing it in a 100 °C water bath. The thus obtained extinction difference is divided by the values of a light-incubated but untreated sample measured in the same way. The values are presented in Fig. 3.

It is clearly seen from the figure that from the values measured at 265 nm we can conclude on the quantitative change of the basic components of the nucleic acids. The quantities of these compounds cannot be expressed in absolute values, since the components are not separately measured, therefore their quantities formed under the influence of light can only be given as a percentage of the untreated sample. When studying the influence of light reactions we expressed the amount of carbohydrates formed from the intermediates under the influence of light also as a percentage of the untreated sample in order to obtain a value comparable to the nucleic base formation.

In determining the photosystem 2 (PS 2) we measured photometrically the reducing effect of 0.1 μM chlorophyll contained in 5 ml solution on the 0.17 μM DPIP. In order to ensure good conditions of measuring we gave the decrease of extinction occurring under the influence of illumination over 5 minutes at an intensity of 100.000 lux in the presence and absence of o. phenanthroline as the inhibitor of PS 2 (Gaffron 1960). When determining the photosystem 1 (PS 1) we measured photometrically the oxidative effect of 0.1 μM chlorophyll on 0.17 μM $DPIPH_2$ (reduced by ascorbic acid) in the presence of o.phenanthroline. The period of illumination had to be 15 minutes at an intensity of 100.000 lux. The quotient of the two light reactions was expressed in an arbitrary unit: $\frac{\text{PS 2 (5 minutes)}}{\text{PS 1 (15 minutes)}}$ - measured in extinction.

We obtained chloroplast suspensions with different light reaction quotients by homogenizing various plants in solutions of different inorganic salt content, with an osmotic pressure corresponding to 0.33 M saccharose. In the course of the examinations a chloroplast suspension containing 0.04 μM/ml chlorophyll was used. The value of the "blank" solution was obtained by acidifying a part of the chloroplast suspension with concentrated sulphuric acid of 1/10 volume immediately after preparation, the placing it in a boiling water bath for 15 minutes and filtering when cooled down. The filtrate thus obtained was used for the further examinations. The so called "untreated" samples were obtained by incubating a part of the chloroplast suspension in dark and another part of it in light of an intensity of 20.000 lux; after incubating we acidified the samples with sulphuric acid the same way as before; then owing to the hydrolysis of starch kept them in a 100 °C water bath for 15 minutes and filtrated. With the view of studying the building up of the intermediates we added $60 \, . \, 10^{-6}$ g/ml of formaldehyde, $250 \cdot 10^{-6}$ g/ml of glycolaldehyde and $500 \cdot 10^{-6}$ g/ml of glyceraldehyde to the chloroplast suspensions, incubated them both in dark and in light and treated them in the above way. The reason why we used different concentrations of the examined intermediaries is that e.g. formaldehyde at a higher concentration than that decreases the value of PS 2; in the case of a formaldehyde concentration of $500 \cdot 10^{-6}$ g/ml no change can be measured in the amount of carbohydrates any longer. Glyceraldehyde at a concentration similar to that of formaldehyde has no influence on the amount of carbohydrates, but a concentration higher than $500 \cdot 10^{-6}$ g/ml may cause troubles during the analytical determination. In studying the building up of carbohydrates a concentration of $250 \cdot 10^{-6}$ g/ml proved to be optimum in the case of glycolaldehyde (with a chlorophyll content of 0.04 μM/ml) (Nosticzius 1973). It seems probable that the ratio of the applied concentrations of these three intermediates is indicative of the ratio of physiological concentrations too. During the examinations the pH of the chloroplast suspension was 7 ± 0.03, which when preparing the inorganic salt solutions used for the homogenization (e.g. NaCl, Na_2SO_4) was fixed with 0.02 M phosphate buffer, or if the inorganic salt solutions contained phosphate the salt solution itself served for buffer. Correlation between the ratio of PS 2/PS 1 and the effect of light is shown in Table 7.

As seen from the data of the table the tendency was the same in the case of all three intermediates. The formation of carbohydrates requires a definite ratio of PS 2 and PS 1, in the case of arbitrary values the optimum is found at 3.25. It is conspicuous, that the formation of nucleic bases changes rhapsodically, and apparently does not demand a definite ratio of PS 2 and PS 1. Another remarkable fact is that from the glyceraldehyde — unlike the formaldehyde and glycolaldehyde — a considerable amount of carbohydrate is formed even in the case of PS 1 dominate, which suggests that this compound is an intermediate of another photosynthetic carbohydrate formation process too. According to literary data (Polya—Osmond 1972, Park—Sane 1971, Andersen *et al.* 1972) the Calvin cycle requires the dominance of PS 1 to come into existence, which suggests that in the case of as low a PS 2/PS 1 ratio as that the glyceraldehyde is built up to carbohydrate through the Calvin cycle. However, the glyceraldehyde must take part — either indirectly or directly — in carbohydrate formation through formaldehyde and glycolaldehyde too, as suggested by the fact that it is optimally built up with the same PS 2/PS 1 ratio as the other two. We obtain a curve with a single optimum even if we use the average of carbohydrate — and nucleic base formation. Fig. 4 shows the mean values of compounds formed from formaldehyde, glycolaldehyde and glyceraldehyde as a response to light. The unbroken line indicates the formation of carbohydrates, the broken line that of nucleic bases, while the hatched area bordered by points represents the average of the two.

Table 7

The extent of photosynthetic carbohydrate and nucleic base formation in plant homogenates, as a function of the ratio between the two light reactions
(Nosticzius 1973)

PS 2/PS 1	Percentage building-up relative to the untreated sample					
	of formaldehyde		of glycolaldehyde		of glyceraldehyde	
	carbohydrate	nucleic base	carbohydrate	nucleic base	carbohydrate	nucleic base
0.20	0.00	0.00	0.00	0.00	7.75	28.82
0.40	0.00	6.16	0.00	5.04	8.64	5.04
0.60	0.00	7.95	0.00	9.80	3.34	9.80
1.00	2.96	4.05	0.81	2.29	1.34	8.45
2.00	0.00	10.43	0.00	4.28	7.46	5.49
3.00	12.27	6.14	10.45	4.36	11.94	26.14
3.25	20.72	9.36	11.71	2.55	44.74	33.82
3.75	16.20	3.16	8.40	2.78	17.37	6.32
4.25	11.34	4.66	0.00	3.36	20.06	6.51
5.00	0.00	9.61	0.00	2.54	0.00	0.66
5.60	1.18	0.00	0.00	0.00	5.39	10.62

We have been thus given the possibility to influence the extent of photosynthesis too, and to study the processes of glycolysis and dark respiration, photorespiration, dark and photosynthetic building up separately, as we can now make any of the processes dominant. We studied the effects of the different ions and found that the anions highly influenced the PS 2 while having no effect on the value of PS 1. Compared to the saccharose the univalent chloride ion increases its value, while the anions with their increasing valency decrease its numerical value (Nosticzius 1973). There is thus a possibility to obtain the optimum value of ratio by homogenizing or resuspending in NaCl solution until producing a concentrated chloroplast suspension. For dilution Na_2SO_4 solution or a buffer prepared from Na_2HPO_4

and KH_2PO_4 can be used. These solutions are best used at concentrations isotonic with 0.33 M saccharose (0.166 M NaCl, 0.111 M Na_2SO_4 and 0.12 M phosphate buffer).

For the quantitative determination of monosaccharides the method of ion exchange column chromatography was used. The borate complexes of monosaccharides were separated on calbiochem AG 1 × 4 resin by gradient elution. As a starting buffer 0.17 M Na-borate of 7.5 pH was used, in this flowed the 8.7 pH 0.444 M Na-borate solution. The hexoses and pentoses, respectively, could be easily separated (Nosticzius 1973) but some hexoses and pentoses came off the column together. This did not, however, disturb the quantitative determination, because hexoses and pentoses next to each other are easy to identify by photometry at 3 walevengths (Nosticzius 1967). After incubation had been completed the liquid (obtained by adding one-tenth volume of concentrated sulphuric acid to the examined solution which then was boiled in a 100 °C water bath for 15 minutes, and filtrated) was neutralized with $CaCO_3$. After filtering and washing it was evaporated dry at 40 °C in vacuum. The sugars were dissolved with pyridine, evaporated dry in vacuum again, and the residue was dissolved in borate buffer required for the chromatographic determination.

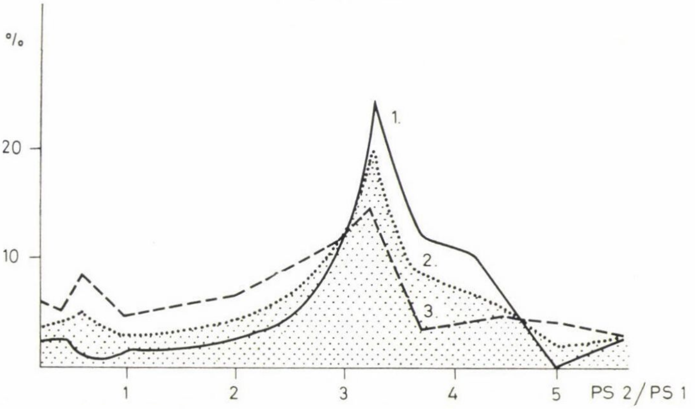

Fig. 4. Building-up in response of light as a function of the PS 2/PS 1 ratio (1 = carbohydrate, 3 = nucleic base, 2 = average of the two)

Dark and photorespiration were also measured on the basis of changes in the amount of carbohydrates only, neither oxygen intake nor carbon dioxide release were measured. Carbohydrate reduction in the untreated sample incubated in the dark relative to the initial value of the so called "blank" solution was regarded as the amount of carbohydrates used up by dark respiration. The reduction of carbohydrates in the untreated sample incubated in light as related to that incubated in dark was considered as the result of photorespiration. The two processes can thus be studied side by side if the ratio between the two processes does not change. In the case of plant homogenates containing many mitochondria the ratio shifts so much in favour of the dark respiration that photorespiration is difficult to measure simultaneously. In Table 8 the results of 14 hours of incubation with lucerne chloroplast suspension are shown.

As seen from the data glucose is the main substrate of both dark and photorespiration. It is conspicuous, however, that more than 90 per cent of the total amount of carbohydrates decreasing with photorespiration consists of glucose. At the same time the amount of ribose increases. This suggests that photorespiration starting from glucose takes place through the pentose-phosphate cycle. As regards the dark respiration, it seems probable that glycolysis and the Szent-Györgyi—Krebs cycle take place simultaneously here, as suggested by the fact

Table 8

*Dark and photorespiration of a lucerne chloroplast suspension containing mitochondria in a contamination-like quantity, on the basis of changes in the amount of monosaccharides**
(NOSTICZIUS 1973)

Monosaccharide corresponding to 10^{-6} g/mg chlorophyll	Blank**	Untreated		Dark-	Photo-
		illuminated	dark	respiration	
Mannose	57	42	67		25
Fructose	176	45	57	119	12
Galactose	199	190	240		50
Glucose	1790	956	1619	171	663
Ribose	71	65	36	35	
Arabinose	131	133	126	5	
Xylose	88	81	86	2	5
Total:	2512	1512	2231	281	719

* PS 2/PS 1 = 3.48
** Initial value
Incubation over 14 hours in light thermostat at a light intensity of 20.000 lux. Sample kept in dark were placed in the same thermostat in a lightproof box

Table 9

Carbohydrate composition of a plant homogenate of low light reaction ratio after 14 hours of illumination and dark, respectively
(NOSTICZIUS 1973)

Monosaccharide corresponding to 10^{-6}g/mg chlorophyll	Blank	Untreated		Formaldehyde		Glycolaldehyde		Glyceraldehyde	
		illuminated	dark	illuminated	dark	illuminated	dark	illuminated	dark
Mannose	45	71	25	54	27	44	103	64	30
Fructose	52	76	73	76	60	47	52	76	42
Galactose	214	294	272	374	383	360	428	335	290
Glucose	1164	483	455	1250	1222	443	569	383	336
Ribose	120	55	42	189	216	166	129	76	57
Arabinose	70	87	120	91	93	133	119	122	100
Xylose	27	41	41	79	82	58	66	59	39
Total:	1692	1107	1028	2113	2083	1251	1466	1115	894

PS 2/PS 1 = 1.33
Incubation over 14 hours in light thermostat. Intensity of illumination: 20.000 lux. Dark samples were placed in a lightproof box

that the decrease of fructose comes near to the extent of glucose reduction while the amount of ribose decreases too, which means that in this experiment the pentose-phosphate cycle has but a minor role in dark respiration. Further on, on the basis of quantitative changes in the individual monosaccharides we can now find out what effects the different intermediates have on the respiration processes. For this purpose we use a plant homogenate containing mitochondria, but adjust the PS 2/PS 1 ratio of the homogenate to a value at which the possibility of photosynthetic building up is minimum. In Table 9 results obtained with a plant homogenate made of barley are shown. The influence exercised by all the three intermediates on respiration is clearly seen. A remarkable phenomenon in this experiment is the inhibition of dark respiration by formaldehyde as suggested by the very high value of glucose measured even in the dark. The fact that the amount of several sugars exceeded the initial — so called "blank" — value in the presence of formaldehyde shows that a dark synthesis also took place in this chloroplast suspension in the presence of formaldehyde, which was nearly of $400 \cdot 10^{-6}$ g/ml chlorophyll value. As another very interesting phenomenon, glycolaldehyde induced

Table 10

*Changes in the total amount of carbohydrates in plant homogenates as a percentage to the untreated sample**
(Nosticzius 1973)

Plant	Carbohydrate decrease in the untreated sample (respiration)	Carbohydrate built up in dark as a response to treatment with		
		formaldehyde	glycolaldehyde	glyceraldehyde
		intermediates		
Pea	0.00	2.54	1.94	15.30
Elder	0.00	0.00	0.00	6.79
Elder	1.58	0.00	0.00	5.61
Lucerne	4.06	1.46	5.85	10.24
Elder	5.07	0.00	0.00	2.72
Pea	7.76	5.82	14.23	13.36
Barley	10.56	9.20	18.98	23.68
Lucerne	17.10	18.42	13.96	19.21
Lucerne	21.05	22.16	12.74	18.56
Lucerne	32.53	32.05	16.91	14.98
Lucerne	36.57	31.30	22.40	19.76
Lucerne	44.54	36.17	24.92	23.68
Lucerne	47.12	35.89	42.73	30.68
Barley	48.06	45.84	15.75	12.44
Lucerne	64.36	65.25	20.94	16.08
Barley	65.21	64.88	22.41	19.40

* Quantitative change of carbohydrates after 14 hours of incubation. Pea, elder and lucerne were grown in the field, the barley in light thermostat

photorespiration which could not be measured in this homogenate without the introduction of glycolaldehyde. It is clearly seen that in the glycolaldehyde treated sample incubated in light the amount of glucose is substantially diminished while the quantity of ribose increases. The extent of dark synthesis is of 400 . 10^{-6} g/ml chlorophyll value in the case of glycolaldehyde too, because this is the difference between the dark incubated sample containing intermediate and the untreated sample incubated in dark. The third intermediate — glyceraldehyde — increased by its presence the intensity of dark respiration, as indicated by a decrease not only in the amount of glucose and fructose but also in the total amount of carbohydrates.

Table 11

*Carbohydrate composition of a plant homogenate containing mitochondria, after 14 hours of incubation in the dark**
(Nosticzius 1973)

Monosaccharide corresponding to 10^{-6}g/mg chlorophyll	Blank	Untreated	Form-aldehyde	Glycol-aldehyde	Glycer-aldehyde
			treated		
Mannose	18	41	52	41	23
Fructose	57	24	49	40	19
Galactose	280	130	181	179	140
Glucose	1373	230	616	247	235
Ribose	262	16	127	14	29
Arabinose	105	66	75	58	42
Xylose	20	28	34	15	52
Total:	2115	535	1134	594	540

* PS 2/PS 1 = 1.87

The fact that glyceraldehyde increases dark respiration is easy to understand when we consider that it is a generally known intermediate of respiration processes. The observation that the presence of glycolaldehyde induces photorespiration suggests that it is an intermediate of this process and its presence probably increases or induces photorespiration through enzyme induction. It remains to be found out whether formaldehyde inhibits all respiration processes, and if so, what the extent of this influence is. For the examination a homogenate of various plants was used. The homogenate was centrifuged at 8000 × g — so that the residue contained the mitochondria too —, then suspended with the solution used for homogenization. All intermediates were used at a concentration of $250 \cdot 10^{-6}$ g/ml. In the case of formaldehyde this concentration exceeded by far the $60 \cdot 10^{-6}$ g/ml value optimum for photosynthesis. By this higher concentration we wished to ensure an increase inhibition. Dark respiration was expressed by the reduction of carbohydrate in the dark incubated untreated sample compared to the initial ("blank") value, while the extent of dark synthesis was established from the difference in carbohydrate content between the dark incubated samples containing intermediates and the dark incubated untreated sample. Insofar as formaldehyde at such a high concentration fully inhibits dark respiration, there must be a linear correlation between dark synthesis and dark respiration. The results are summed up in Table 10.

As seen from the results it is only in the case of the formaldehyde intermediate that any correlation exists between dark respiration and dark synthesis. However, the correlation is not quite linear even with such a high formaldehyde concentration, which suggests that not all of the respiration processes are inhibited by it. From this point of view the experiment in which barley seedling was homogenized with 0.166 M NaCl solution containing 0.01 M cysteine was interesting. For resuspending after centrifuging at 8000 × g a NaCl solution no longer containing cysteine was used. In the course of incubation the test tubes furnished with glass stoppers were filled to repletion with the homogenate, so that during the incubation there was no air present above the suspension and the applied cysteine could reduce its oxygen content substantially. Under such conditions glycolysis became independent of the Krebs—Szent-Györgyi cycle as proved by the very great extent of carbohydrate reduction too. The results are shown in Table 11.

Table 12

*Monosaccharides formed from the intermediates in elder chloroplast suspension**
(Nosticzius 1973)

Monosaccharide corresponding to 10^{-6}g/mg chlorophyll	Blank**	Untreated		Formaldehyde		Glycolaldehyde		Glyceraldehyde	
				treatment					
		illuminated	dark	illuminated	dark	illuminated	dark	illuminated	dark
Mannose	160	133	14	128	123	95	195	160	198
Fructose	498	291	247	274	456	284	458	393	491
Galactose	602	833	560	1 435	609	1 133	486	1 007	719
Glucose	9 317	9 207	9 605	10 877	9 261	9 933	8 265	10 980	9 263
Ribose	100	95	63	56	17	109	58	302	133
Arabinose	40	102	107	135	40	91	119	179	198
Xylose	40	53	67	84	37	81	44	277	42
Total:	10 757	10 714	10 663	12 989	10 543	11 726	9 625	13 298	11 044
Photosynthetic growth of total carbohydrates		51		2 446		2 101		2 254	

* Incubation over 14 hours in light thermostat; the dark samples were placed in a light-proof box

PS 2/PS 1 = 3.36

** Initial value

Treatments: formaldehyde $60 \cdot 10^{-6}$g/ml; glycolaldehyde $250 \cdot 10^{-6}$g/ml; glyceraldehyde $500 \cdot 10^{-6}$g/ml

Chlorophyll 0.04 μ mol/ml

According to the data of the table formaldehyde is not able to inhibit either the glycolysis or the pentose-phosphate cycle (as indicated by the increased quantity of ribose too); it merely inhibits the Krebs—Szent-Györgyi cycle.

As seen from Table 10, in elder the respiration processes are reduced to a minimum, so a chloroplast suspension prepared from this plant seemed to be the most suitable for carrying out investigations on photosynthesis.

The elder leaf was only homogenized for 30 seconds then centrifuged for 2 minutes at 600 × g to remove the intact cells. The supernatant was centrifuged again at 1500 × g for

6 minutes. The sediment was resuspended with 0.166 M NaCl solution of pH 7 containing 0.02 M phosphate as buffer, the same as used for the homogenization. The chloroplast suspension was diluted with 0.12 M phosphate buffer of adequate ratio in order to approximate the 3.25 PS 2/PS 1 value earlier found to be optimum. The results are shown in Table 12.

According to the data of the table nearly identical quantities of carbohydrates were produced photosynthetically from the three intermediates. Photosynthetic building-up was in all three cases somewhat more than 2 thousand gamma/mg chlorophyll total carbohydrates (gamma = μg). On account of using chloroplasts not containing mitochondria it was only in the case of glyceraldehyde that dark synthesis occurred which was never observed — because of accelerated respiration — in the case of homogenates containing mitochondria. The extent of dark synthesis is 381 gamma/mg chlorophyll when expressed in total carbohydrates, which is in full agreement with the nearly 400 gamma quantity earlier obtained with formaldehyde and glycolaldehyde treatments. This is the highest value of photosynthesis measured by us so far, and it is almost identical with the three intermediates. The extent of dark synthesis is also nearly the same as in the case of formaldehyde and glycolaldehyde, which means that the dark synthesis amounts to almost 20 per cent of the photosynthetic carbohydrate formation if the latter occurs from these three intermediates.

That the pathway of synthesis is identical for the three intermediates is suggested by the fact that identical monosaccharides were built up from them under the influence of light. The photosynthesis of galactose, glucose and ribose took place in the case of each of the three intermediates; the main product was glucose in all cases.

We may obtain some information about the course of synthesis if we examine the changes in the monosaccharides in dark and light in the untreated and intermediate treated samples. Changes relative to the "blank" value are presented in Table 13.

The quantity of fructose decreases both in the untreated samples and in those treated with intermediates; this decrease was, however, substantially lower in the latter. The decrease of fructose becomes nearly identical with the value of the untreated sample under the influence of light. The observed phenomenon can be explained in two ways. The intermediates may be supposed to inhibit the glycolysis, and this inhibition is topped by light. There is little likelihood of this process. The most probable explanation is the formation of fructose from the intermediates in the dark with the help of ATP. The fructose content of the untreated sample was transformed to glucose in the dark in the presence of ATP as proved by the fact that the glucose content of the untreated sample increased almost at the same rate as the fructose content decreased. In the case of samples treated with intermediates the rate of fructose decomposition increased under the influence of light because the photosynthetically produced ATP rendered it possible for the fructose to transform into glucose. This means that the formation of fructose precedes that of glucose.

The addition of glyceraldehyde in all probability sets in motion the Szent-Györgyi—Krebs cycle which starts from glyceraldehyde and not from carbohydrates. This is suggested by the fact that as a response to glyceraldehyde treatment the transformation of many carbohydrates occurs even in dark, while in the presence of the other two intermediates it only takes place in light. This can be explained by the starting oxidation processes supplying energy that the other two intermediates are only able to supply in the presence of light. From glyceraldehyde galactose is produced even in the dark, while glucose is not. Considering the untreated samples we find, on the other hand, that when illuminated the extent of fructose reduction agrees with the rate of increase of galactose rather than with that of glucose. This means that the galactose is also produced from fructose (perhaps through glucose).

The galactose contents of both the untreated samples and those treated with intermediates decrease in the dark — except for the special case of glyceraldehyde mentioned above —, at the same time the amount of arabinose generally grows. Under the influence of

Table 13

Change of monosaccharides in a mitochondria-free elder chloroplast suspension relative to the value of the blank sample
(Nosticzius 1973)

Monosaccharide corresponding to 10^{-6}g/mg chlorophyll	Change relative to the blank				Change as a response to light		Treatment*
	in light		in dark				
	increase	decrease	increase	decrease	increase	decrease	
Mannose	—	27	—	146	119	—	U
	—	32	—	37	5	—	F
	—	65	35	—	—	100	Gk
	—	—	38	—	—	38	Gc
Fructose	—	207	—	251	44	—	U
	—	224	—	42	—	182	F
	—	214	—	40	—	174	Gk
	—	105	—	7	—	98	Gc
Galactose	231	—	—	42	273	—	U
	833	—	7	—	826	—	F
	531	—	—	116	647	—	Gk
	405	—	117	—	288	—	Gc
Glucose	—	110	288	—	—	398	U
	1560	—	—	56	1616	—	F
	616	—	—	1052	1668	—	Gk
	1663	—	—	54	1717	—	Gc
Ribose	—	5	—	37	32	—	U
	—	44	—	83	39	—	F
	9	—	—	42	51	—	Gk
	202	—	33	—	169	—	Gc
Arabinose	62	—	67	—	—	5	U
	95	—	—	—	95	—	F
	51	—	79	—	—	28	Gk
	139	—	158	—	—	19	Gc
Xylose	13	—	27	—	—	14	U
	44	—	—	3	47	—	F
	41	—	4	—	37	—	Gk
	237	—	2	—	235	—	Gc

* U = untreated; F = formaldehyde; Gk = glycolaldehyde; Gc = glyceraldehyde

formaldehyde the galactose content is not reduced nor does the amount of arabinose grow. In the case of glyceraldehyde an intensive growth of the amount of galactose may occur in the dark too, that is why the quantities of both galactose and arabinose increased in our experiment. The fact that in the presence of formaldehyde the amount of galactose is not reduced and the arabinose content does not increase suggests that the relatively high reducing effect of formaldehyde inhibits the transformation. One of the pathways of arabinose formation is the decarboxylation of galacturonic acid. One of the ways xylose is produced is through the decarboxylation of glucuronic acid. In the presence of formaldehyde xylose failed to increase in the dark the way arabinose did. Under the influence of light the formation of both arabinose and xylose starts in the samples containing formaldehyde. It seems to be probable that one of the main pathways these two pentoses are formed through — under the given conditions — is the decarboxylation of the proper uronic acids.

It may be of decisive importance to explain the quantitative change of ribose. We may start from the fact that from the glyceraldehyde ribose is formed even in the dark, and the energy required for this is in all probability supplied by high energy compounds produced during the reactions of the Szent-Györgyi—Krebs cycle that starts under the influence of glyceraldehyde. The possible reason why in the dark ribose is not formed from the other two intermediates is the absence of high energy compounds ensuring the process of building-up. In light, ribose is formed from all the three intermediates. In dark, on the other hand, the amount of ribose decreases in the case of both formaldehyde — and glycolaldehyde treated samples, while in the sample treated with glyceraldehyde — for reasons already mentioned —, the quantity of ribose increases. This proves that the hexoses are produced through the pentose-phosphate cycle.

The fact that from the three intermediates the same product — glucose — is formed on the one hand, and that from all of them a considerable amount of galactose is produced, on the other hand, further, that the rate of total carbohydrate synthesis is nearly identical shows the same building-up reaction for all the three intermediates. Differences between the quantities of monosaccharides produced — e.g. the relatively high pentose content caused by the presence of glyceraldehyde — can be regarded as the latter entering the same process at a different point than the other two intermediates.

Table 14 shows the hexose — and pentose contents as well as the ratio of the two in the different samples. The oligosaccharide hexose included in the table contains the values of maltose. In the course of the sulphuric acid hydrolysis, starch is only decomposed to maltose and not to glucose, so the oligosaccharide value can be regarded as starch. Hexose built up to starch probably has no influence on the ratio of hexose and pentose, that is why we presented the hexose-pentose ratio of monosaccharides too.

Glyceraldehyde — at the concentration used by us — shifts the ratio of hexose to pentose in a great measure in favour of pentose formation, especially under the influence of illumination. Formaldehyde — particularly in the dark — sharply reduces the amount of pentoses compared to that of hexoses. The fact that as a response to illumination the hexose—pentose ratio decreases again indicates the photosynthetic formation of pentose from formaldehyde. From glycolaldehyde pentoses were formed in a more or less natural proportion.

The formation of nucleic bases is shown in Table 15. Values expressed in adenine were only considered on the basis of extinction measured at 265 nm. It is clearly seen from the data that from the glyceraldehyde intermediate considerable amounts of nucleic bases were formed both in the dark and in light. Taking into consideration the formation of pentoses such a high rate formation of nucleic bases becomes easy to understand, as phosphoribosyl pyrophosphate is the initial material of nucleic base formation.

The fact that formaldehyde and glycolaldehyde are built up to carbohydrates in a similar way (through glycolaldehyde), while this process is somewhat different in the case

Table 14

Changes in the ratio of hexoses to pentoses as a reaction to treatments with intermediates in elder chloroplast suspension
(Nostitzius 1973)

Carbohydrate corresponding to 10^{-6}g/mg chlorophyll	Blank	Untreated		Formaldehyde		Glycolaldehyde		Glyceraldehyde	
				treatment					
		illuminated	dark	illuminated	dark	illuminated	dark	illuminated	dark
Total hexoses	10 577	10 464	10 426	12 714	10 449	11 445	9 404	12 540	10 671
Monosaccharide hexose	5 623	8 899	8 479	10 877	8 026	9 840	8 162	11 119	9 471
Oligosaccharide hexose	4 954	1 565	1 951	1 837	2 423	1 605	1 242	1 421	1 200
Pentose	180	250	237	275	94	281	221	758	373
* H/P in total carbohydrates	58.76	41.86	43.99	46.23	111.16	40.73	42.55	16.54	28.61
* H/P monosaccharide	31.24	35.60	35.76	39.55	85.38	35.02	36.93	14.67	25.39

* H/P = hexose per pentose ratio

of glyceraldehyde is supported by the extinction values obtained at 340 nm too. The value obtained at 340 nm is a figure characteristic of the amount of reduced state pyridine nucleotides, that is it can be used for measuring the quantities of $NADH_2$ and $NADPH_2$. As to the absolute values, we cannot draw far-reaching conclusions, but the nature of change is well perceptible. It is quite clear from the data of Table 16 that in the untreated sample the amount of reduced pyridine nucleotides increased through photosynthesis, and since it was not able to utilize the thus produced $NADPH_2$ their quantity remained unchanged.

The formaldehyde and glycolaldehyde even used up a part of the originally present reduced pyridine nucleotides, while the glyceraldehyde still increased their quantity. Since in the elder chloroplast suspension mitochondria is practically not present, only the presence of NADP of the pyridine nucleotides has to be reckoned with, because NAD is mostly found in the mitochondria. The Szent-Györgyi—Krebs cycle does not take place, so only the reduction or reoxidation of NADP must be taken into account.

Table 15

Nucleic bases formed from the intermediates in elder chloroplast suspension

(Nosticzius 1973)

Nucleic base equivalent to 10^{-5}g adenine/mg chlorophyll	In light	In dark	Change			
			compared to the untreated		under the influence of light	
			in light	in dark	increase	decrease
Blank*	28.94					
Untreated	31.48	30.48				
Formaldehyde	31.03	30.78	—0.045	0.30		0.345
Glycolaldehyde	33.85	34.94	2.37	4.46		2.09
Glyceraldehyde	54.03	46.58	22.55	16.10	6.45	

S.D. 5% = 1.54
* Initial

The increased quantity of pentoses is but partly responsible for the amount of $NADPH_2$ increasing under the influence of glyceraldehyde. If the pentoses are produced by the direct oxidation of glucose, as oxidation here is affected by a NADP specific enzyme an oxidation into NADP can be imagined.

On the other hand, the amount of pentoses grew in the untreated and glycolaldehyde treated samples kept in the dark, while the extinction values measured at 340 nm decreased or hardly changed. The latter values prove that formaldehyde and glycolaldehyde are built up to carbohydrate in the same way, during the synthesis $NADPH_2$ is oxidized; when glyceraldehyde is built up to carbohydrate a different character reaction occurs as well, in the course of which the NADP is reduced.

Summing up the information given so far we tried to integrate our own experiences with other authors' observations and with the known reactions of carbohydrate metabolism. However, the explanation of other authors' data often differs from the authors' original explanation.

Table 16

Thousandfold extinction values measured at 340 nm in elder chloroplast suspension
(Nosticzius 1973)

Treatment	In light	In dark	Change			
			relative to the blank		under the influence of light	
			in light	in dark	increase	decrease
Blank	268					
Untreated	288	262	20	−6	26	
Formaldehyde	236	263	−32	−5		27
Glycolaldehyde	252	270	−16	2		18
Glyceraldehyde	301	308	33	40		7

S.D. 5% = 11.2

Discussion

1. According to Baldry *et al.* (1966) and O'Neal *et al.* (1972) the introduction of non-radiating glycolaldehyde and glycolaldehyde phosphate reduces the photosynthesis from radiating $C^{14}O_2$. According to our experimental results a light dependent formation of carbohydrates from glycolaldehyde added to the chloroplast suspension took place; the hexose–pentose ratio of the produced carbohydrates was very near to the original hexose–pentose ratio of the chloroplast suspension. To explain the observation of the mentioned authors: in the case of glycolaldehyde and its phosphate added the decrease in the intensity of photosynthesis from radiating $C^{14}0_2$ is caused by a light-dependent building up of non-radiating glycolaldehyde to carbohydrate.

2. According to Schacter *et al.* (1971) the photosynthetic activity reduced with the inhibition of the Calvin cycle will be restored when fructose-1.6-diphosphate or ribose-5-phosphate or glyceraldehyde-3-phosphate is introduced into the system. This means that in this case with the help of these three compounds a product begins to form which indirectly is suitable to take up CO_2 without the formation of Ru-1.5-DiP. According to our own experiments as a response to glycolaldehyde added to a chloroplast suspension and with incubation in dark the concentration of fructose increases and that of the pentoses slightly decreases. This suggests that the glycolaldehyde produces fructose indirectly at the expense of pentoses. The experience of Datta—Racker (1961) allows glycolaldehyde to be built up to a six-carbon carbohydrate if we assume that the reaction observed by them can be reversed. They found that from Fr-6-P a complex of these two compounds was produced by transketolase. According to further experiences obtained by Bradbeer—Racker (1961) the thus produced glycolaldehyde was oxidized even by ferricyanide to glycolic acid. The reaction was:

$$\text{Fr-6-P} + \text{trandketolase} \rightleftharpoons \text{Fr-6-P} - \text{transketolase}$$

$$\text{Fr-6-P} - \text{transketolase} \rightleftharpoons \text{Er-4-P} + \text{glycolaldehyde-transketolase.}$$

This finding was confirmed by Shain—Gibbs (1971) who found the transketolase added to spinach chloroplast to produce glycolic acid from Fr-6-P in a light dependent way. Thus the glycolaldehyde-transketolase complex was the first to break off. Glycolaldehyde is likely to be built up to fructose through the reverse of this reaction. The supposed reaction of incorporation is thus

Er-4-P + glycolaldehyde-transketolse → Fr-6-P + transketolase.

The phenomenon observed by SCHACTER *et al.* (1971) can be interpreted as these three compounds contributing to the formation of Er-4-P which plays the part of the acceptor, promoting thereby the intensity of photosynthesis. There is a possibility of this according to our present knowledge. The role of Fr-6-P and glyceraldehyde-3-P can be simultaneously seen if we consider the reaction catalyzed by the transketolase:

Fr-6-P + glyceraldehyde-3-P ⟶ Xu-5-P + Er 4 P

The role of Ri-5-P is also clearly seen if we consider first the reaction catalyzed by phosphoriboisomerase

$$\text{Ri-5-P} \rightleftharpoons \text{Ru-5-P} \rightleftharpoons \text{Xu-5-P}$$

then that by transketolase

Xu-5-P + Ri-5-P ⟶ Se-7-P + glyceraldehyde-3-P

and finally the reaction catalyzed by transaldolase

Se-7-P + glyceraldehyde-3-P ⟶ Fr-6-P + Er-4-P

There are more than one possibilities of glycolaldehyde being synthetized by transketolase to carbohydrate. According to the reaction published by PROCHOROFF *et al.* (1962) it may be built up to carbohydrate through the process of

Ri-5-P + glycolaldehyde-transketolase ⟶ Se-7-P + transketolase.

Moreover, through the process discovered by HORECKER *et al.* (1956) it can take part in producing Xu-5-P too. The decrease of pentose and increase of fructose in dark incubated samples treated with glycolaldehyde suggest a decisive role of Fr-6-P formation from Er-4-P and Se-7-P formation from Ri-5-P in the uptake of glycolaldehyde. When seen in this new light the stimulatory effect on photosynthesis of Fr-1.6-DiP, glyceraldehyde-3-P and Ri-5-P becomes easy to understand. With the inhibition of the Calvin cycle the otherwise not dominant reaction of photosynthetic carbohydrate formation through glycolaldehyde becomes effective.

The above mentioned are well illustrated by a table in a repeatedly cited paper of SCHACTER *et. al.* (1971).

When we look at the column of glycolate formation in the table we can clearly see that in the presence of Fr-1.6-DiP, Ri-5-P and Fr-6-P alike the amount of glycolic acid produced under the influence of light considerably decreases compared to the control. This can be perfectly explained by the fact that these three compounds are able either directly or indirectly (by changing into Er-4-P) to uptake the photosynthetically formed glycolaldehyde. If the amount of this latter decreases, the quantity of glycolate formed from it will decrease too.

The above listed reactions render it possible for us to assume the existence of a cycle which does not demand the formation of Ru-1.5-DiP.

3. As the behaviour of formaldehyde was very similar to that of glycolaldehyde during the photosynthetic carbohydrate formation we have to suppose that the glycolaldehyde is produced from two formaldehydes in the course of photosynthesis. (The fact that in the case of the dark incubated, formaldehyde treated sample the hexose-pentose ratio increases does not mean at all that the formaldehyde can be built up to carbohydrate without changing first into glycolaldehyde, since the total amount of carbohydrate did not grow. When illuminated, on the other hand, it was built up to carbohydrate almost in the same measure as glycolalde-

hyde which suggests that the two formaldehydes also need the presence of light to be synthesized to glycolaldehyde.)

4. From the three examined intermediates carbohydrates were formed in almost identical quantities which shows that formaldehyde, glycolaldehyde and glyceraldehyde are intermediates of full value in the new pathway of photosynthetic carbohydrate formation. Formaldehyde and glycolaldehyde formed carbohydrates in nearly identical proportions almost true to nature, while glyceraldehyde — though having become a main product of the same value — promoted the formation of pentoses. We have to suppose, therefore, that glyceraldehyde also takes part in the new process, only enters the cycle at a different point.

5. On the basis of what have been described so far the scheme of the photosynthetic cycle is presented in Fig. 5.

Table 17

Effect of some intermediates of the photosynthetic carbon reduction cycle on the distribution of $^{14}CO_2$ assimilated

Incubation was carried out in the standard reaction mixture under N_2 with $NaHCO_3$ at 5 mM. The sugar phosphates were added as indicated to give a concentration of 1 mM. The samples used for paper chromatography were taken 10 minutes after turning on the light. (Schaceter *et. al* 1971).

	Control	Fructose 1,6-diP	Ribose 5-P	Fructose 6-P
	% total cpm fixed			
Insolubles + sugar diphosphates[1]	16.7	17.5	21.0	15.9
Sugar monophosphates[2]	5.1	4.4	7.7	16.4
Glycerate-3-P	14.1	17.3	21.7	21.1
Triose phosphates[3]	56.3	57.5	46.2	42.9
Glycolate	8.0	2.9	3.4	3.7
Rate of fixation μmoles/mg chl-hr	42	60	64	42

[1] Polyglucan, ribulose diphosphate and fructose diphosphate
[2] Glucose and fructose.
[3] Essentially dihydroxyacetone-P, some glyceraldehyde 3-P

6. The question involuntarily arises what the reason of the low perceptibility of this new process of photosynthetic carbohydrate formation compared to the Calvin cycle and the Hatch, Schlack, Kortschak process is. Most probably it is only a definite group of the photosynthetizing cells that builds its carbohydrates in this way. A number of observations confirm that this form of photosynthesis has a part in the stomatal function. Zelitch—Walker (1964) and Zelitch (1969) studied the relationship between the formation of glycolic acid and the function of stomata and found a close correlation between the two processes. Raschke (1972) pointed out a correlation between carbon dioxide concentration and stomatal functioning, while Akita—Moss (1973) proved the relation of the presence of oxygen to stomata opening. The two latter findings give evidence of a correlation between the preconditions of photosynthetic glycolic acid formation and stomatal functioning. The uptake of cations (K^+, Rb^+) may be supposed to be a secondary phenomenon, a mere consequence of the acidic pH, and that is why a correlation can be found between their uptake and the stomatal functioning.

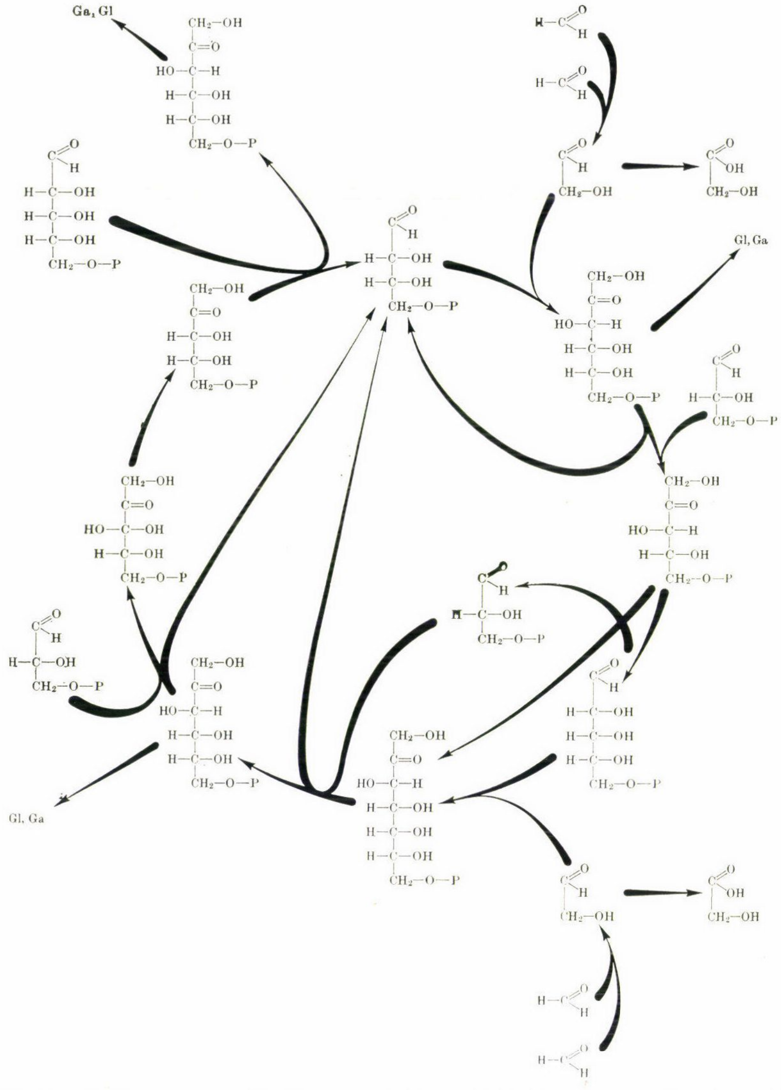

Fig. 5. Biochemical processes of photosynthetic carbohydrate formation through glycolaldehyde

It seems, however, probable that all photosynthetizing cells are able to build up their carbohydrates through this pathway of photosynthesis, but which of the three possible processes will be dominant is determined by the PS 2/PS 1 ratio of the chloroplast — that is, in fact, by the redox potential. The Calvin cycle is completed with the dominance of the PS 1 too, therefore this can be perceived with the highest probability. According to the literature on light reactions the Hatch, Schlack, Kortschak process takes place when the PS 2 is domineering, while the demand on the ratio of PS 2/PS 1 of the process of carbohydrate formation through glycolaldehyde is between the two. The inhibition of one of the processes and introduction of an intermediate required for the other may start or enhance the other process, which otherwise plays a minor role.

It is also interesting from this point of view that the perceptibility of the new process is higher at an advanced stage of the plant (after flowering). With young barley plants grown in a light thermostat it can be pointed out that from the point of view of perceiving the new process the optimum way of raising the plants is the alternation of 12 hours dark and 12 hours light periods. Whether we prolongate the period of illumination or increase the length of the dark period, the amount of carbohydrates formed from formaldehyde and glycolaldehyde will decrease in the chloroplast.

May this paper excite interest and induce other researchers to take this new possibility into consideration when evaluating and explaining their own results. If it succeeds in calling attention to the viewpoints listed here the cycle shown in Fig. 5 may become an established fact; for any hypothesis only becomes proved when a number of researchers using different methods arrive at the same conclusion.

Abbreviations

CAM: Crassulacean acid metabolism; DiP: diphosphate; DPIP: 2.6-dichlorophenolindophenol; Er: erythrose; ext. (extinction, value of photometric measuring): $-\log \frac{I}{I_0}$; Fr: fructose, P: phosphate; Ri: ribose; Ru: ribulose; Se: sedoheptulose; Xu: xylulose.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 209—230 (1976)

CONTRIBUTIONS

to the paper of F. Radics: "Statistical comparison of the angles of main ribs in the leaves and leaf remnants of *Platanus acerifolia* (Ait.) Willd. and *Platanus aceroides* (Goepp.) Heer (fossil)" published in this periodical, *24*, (1—2).

CAN THE HYBRID ORIGIN PROGENY OF PLANE-TREES REPRESS THE BASIC SPECIES IN TWO CENTURIES?

The philosophers of ancient Athens used to walk under plane-trees; it was they who gave the platan the name "philosophers' tree", and regarded it as a symbol of contemplation. The cult of the platan was taken over by the Romans who passed it on to Europe — like all of their culture — through Gaul. The platan species in question was the one with moderately lobed leaves providing more shade, that has been called *Platanus acerifolia* up to the present day.

The western platan was brought to Europe from America in the 17th century at the earliest — according to Furber before 1724 — and spread at about the turn of the 18th and 19th centuries. The crossing of the two platans — *P. orientalis* and *P. occidentalis* — is biologically impossible, and even if a hybridization had occurred, a uniform progeny could not have spread in such a great mass during the past two centuries as to repress the basic species to the extent this has happened.

In Hungary the oldest and bulkiest plane-tree is found in the former Batthyány park at Körmend; its diameter at breast height is 2.48 m. Owing to its huge, 50 m diameter crown, closed to the ground, its annual rings are very thick. According to the report of Károly Kaán (1931) it was 6 m in circumference, that is of 1.91 m diameter at breast height 45 years ago. At present its perimeter is 7.80 m (that is its diameter 2.48 m). The thickness of its annual rings is 0.65 cm, thus the tree must have been planted only 200 years ago, at the end of the 18th century. It cannot be a hybrid of European and American plane-trees.

After the Napoleonic wars a quiet period followed in Europe which was favourable for such peaceful occupations as landscape architecture too. At the beginning of the 19th century platan and horse chestnut were the most favoured tree species of the parks established around the Hungarian manor-houses (pines came only later). The famous alleys at Nagycenk and Alcsut as well as the forest-like platan plantations at Mosonmagyaróvár and Szikra originate from the same period and have been known so far as permanent hybrids of *Platanus acerifolia.*

Ferenc Radics's statement puts an end to the confusion. The historical relations of platan plantations found in the parks and forests prove that the Hungarian plane-tree cannot be an European–American hybrid; it is an old South European species widely introduced in Central Europe mainly at the beginning of the 19th century when landscape architecture flourished.

The plane-tree has numerous advantages which justify its increasing forestry cultivation. It grows rapidly on the flood areas of Hungary, and in a closed stand yields a fine, long, cylindrical log. Its hard, beech-like wood is much sought-after in the carpenter and furniture industry, and is an excellent paper and fibre wood. Some of its clones can be propagated from cuttings too, and its seedlings are easy to raise. It has a high aesthetic value. It has the particularly favourable property of being attacked by hardly any pest or disease. Even the game does not damage it. For this very reason under the warm and wet conditions of our flood areas rich in game we are compelled to use platan instead of poplar. After the development of an appropriate commercial variety an extensive forestry utilization of plane-trees can be expected. All this makes the exploration of its genetic properties urgent. It is the basic and practical research of forest plant breeding that will finally solve the platan problem. For the meantime, we can agree with Radics's opinion formed by the biometric comparison of the angles of leaf main ribs, and instead of regarding the plane-tree growing in Hungary too as a hybrid accept it as an ancient European platan, a variety of *Platanaus aceroides* (Goepp.) Heer.

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DOES HETEROPHYLLY OCCURRING DURING THE ONTOGENESIS OF THE PLATANUS SPECIES GIVE ANY INFORMATION ON THE ORIGIN OF THE MEDITERRANEAN PLATANS?

The differences in the angles enclosed by the main rib and the lateral veins of *Platanus acerifolia* leaves suggest the idea that during the ontogenesis the leaves first become more and more differentiated, then a simplification follows. This can be found when the shoot is formed in the course of germination, too, and the first foliar leaves are to some extent similar to the cotyledons while the leaf type characteristic of the species appears afterwards, but the same can be observed on the leaves of long shoots developing from buds. Leaves immediately above the scales of the leaf-bud are simple and resemble the scales, while those higher up show an increasing differentiation; near to the apex again simpler leaves develop. To a certain extent this is connected with the nutrient conditions, the development stage of the vascular system and the nutrient abstraction by the lower leaves.

Heterophylly appearing during the ontogenesis of plants can be observed on the shoots of the platan species too; first the leaves are more or less ovate, alate, with pinnated veins, then the lower lateral veins become stronger, move further away from those above them and a palmate form of leaves and vein system appears. These leaves developing on the lower and upper parts of the shoots are generally smaller too. The angle of the main rib is much smaller, while towards the middle of the shoot it increases until attaining the value characteristic of the species. We present here the left and right-side angles formed by the main rib on leaves of different development stage of *Platanus acerifolia*.

	Left-side	Right-side
1	31°	32°
2	34°	35°
3	43°	41°
4	45°	47°

These leaves which are smaller in size and have smaller main rib angles are found in about 15—25 per cent. Most leaves are, however, of a shape and size characteristic of the species with main rib angles belonging to category 4 in the above Table.

In the case of *Platanus occidentalis* and *orientalis* leaves with a pinnate vein system and smaller main rib angles occur with a similar frequency, but the bulk of the leaves has attained full differentiation.

Differences between the species in the size of the angles of the main rib are rather considerable. The main rib angles of *Platanus orientalis* leaves are much smaller than those in *Platanus acerifolia* (not to mention the 48—69° angles of *Platanus occidentalis* leaf ribs already discussed in the paper), so these platans are considered to belong to different species.

It is remarkable how much the leaves of *Platanus occidentalis* and *P. orientalis* differ from each other, besides both differing from the leaf type of the recent *P. acerifolia* and ancient *P. aceroides*. It can be supposed that the *Platanus* first described from the "koma" series of the Greenlandic lower cretaceous period came under different influences during its subsequent distribution — which took place in an east-west direction — and leaf types rather different both from the ancient form and from one another developed (in the case of *Platanus occidentalis* and *P. orientalis*), while the recent *P. acerifolia* remained for a longer time in or near the site of origin of the ancient platan under nearly identical ecological conditions at the time when the changes occurred, so its leaf properties hardly changed, and when spreading over larger areas maintained a high similarity to *P. aceroides*.

Furthermore, according to our present knowledge in the cretaceous period the connection of Greenland westward with America and eastward with Europe and Asia was still closer. From then on the continents gradually separated and the distribution area of *Platanus* increased. In the burdigala and helvet layer of the micene leaf remains of the ancient *Platanus* have already been pointed out in Hungary too (Transdanubia and the neighbourhood of Eger). Thus the evolution of *P. occidentalis* and *P. orientalis* must have taken place later. Further investigations are expected to decide when the *P. occidentalis* and *P. orientalis* appeared, in which geohistorical periods their remains can be pointed out and how they are related to the finds and time of appearance of *P. aceroides* and *P. acerifolia*.

From a morphogenetic point of view this question raises a further problem, and may approach the solution, namely the development of the palmate leaf. As mentioned before on some shoots the shape and venation of the leaves show a considerable diversity. From the initial pinnate pattern of leaves and veins a gradual transition into palmate leaves and venation can be observed. This seems to confirm that the palmate leaf developed from the pinnate form through the intensive growth and elongation of the lateral veins in the basal part of the leaf blade which resulted in the larger size and palmate form of the leaf. This development of broader leaves may have been connected with the warm and moist conditions of the environment. The increasing leaf surface involved a more intensive evaporation but at the same time a more intensive assimilation too. This is largely supported by the fact that in the Greenlandic layer a flora composition resembling the vegetation of the moderate and tropic zones has been pointed out *(Magnolia, Launes, Cinnamonum, Platanus, Quercus, Populus* primaers, *Astocarpus, Delbergites).*

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IS THE HISTORY OF THE PLANE-TREE CULTURE TO BE REWRITTEN?

In his paper Ferenc Radics starts from the supposition that the *Planatus acerifolia* widely grown in European parks and alleys is not of hybrid origin but a direct descendent of the ancient tertiary *P. aceroides* that has survived in human culture. To support his theory a statistical comparison is given in his paper between the recent and fossil taxa concerned. Accepting the similarity as being satisfactory, he rejects the possibility of hybrid origin.

Platanus species have for a long time been classic examples of the allopatric origin of species which did not involve the development of a genetic barrier. On the basis of the book of Clausen—Hiesey (1958, pl. 246—247), the classical work of Stebbins (1950, p. 199—200) as well as the Sarmatian flora (Andreánszky 1959) known and referred to by the author himself, the picture is clear. The area of the tertiary *P. aceroides* was broken up by the glaciation giving rise to *P. orientalis* (sensu lato) in the Eastern-Mediterranean regions and on the areas of Asia Minor extending to the Himalaya. Similarly, in the eastern part of North America *P. occidentalis* and in the warmer western parts *P. racemosa* evolved.

P. orientalis adapted itself to areas with mild winters. The Romans cultivated it in many places, but with the extension of the Roman Empire it could not get far to the North owing to its frost sensitiveness. *P. occidentalis*, on the other hand, is well adapted to the hard winter.

According to *earlier rceords.* (Henry—Flood 1919) *P. occidentalis* and *P. orientalis* were planted in England *in the* Oxford Botanical Garden in the middle of the 17th century. Here the two species hybridized spontaneously around 1670. The hybrid proved to be a viable fertile plant with normal meiosis (Winge 1917, Sax 1933), and its seed progeny (F_2) showed a varying segregation of the characters of the two parents. Partly these, partly the vegetative progeny of the F_1 hybrid formed the basic material of the popular plane-trees of European parks, since they combined the favourable properties of the two parent species, and above all showed a high frost tolerance.

If the above outlined picture is to be radically changed — as proposed by Ferenc Radics — a suitable method of disproving the hybrid origin must be chosen.

If during the evolution two new species have arisen from an ancestral one as a result of different adaptation, it is natural that the hybrid of these new species will show a high similarity to the initial form. So the morphological measurements of leaves performed by the author — no matter how precisely they are carried out — do not help in answering the question of hybrid origin. More convincing proofs can be obtained by either of the following two ways:

a) The author himself mentions that plane-tress are long-lived, they may even live for 2000 years. He could thus disprove the hybrid origin by finding a specimen of *P. acerifolia* which is older than 400 years of age. (*P. occidentalis* is not supposed to have been brought into Europe earlier than the 17th century.)

b) It is a well known fact that the seed progenies of hybrids (unless raised to polyploidy or able to multiply in an apomictic way) show remarkable segregation. Thus, provided *P. acerifolia* is a hybrid of *P. orientalis* and *P. occidentalis*, the leaf index variation found in the progeny should overlap with that of *P. orientalis* and *P. occidentalis.* On the other hand, if it is not a case of hybrid origin, the diversity of the seed progeny is low, and does not differ in order of magnitude from the seedling variation range of the pure species of *P. orientalis* or *P. occidentalis.*

If by any of the above two methods the author obtains new evidence of the non-hybrid origin of *P. acerifolia*, then the rewriting of the history of the European plane-trees may really become necessary.

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WHY NOT USE A SIMPLE ANALYSIS OF VARIANCE?

The author tries to answer a question of taxonomy and paleobotany, the taxonomic position of the fossil species *Platanus aceroides*, by means of a comparison with recent species, using the methods of quantitative biology. First he tries to find useful characters, to describe them in quantitative terms, and then to solve the problem on this basis with the help of biometric tests. The author can prove that the measurements of the fossil remnants of *P. aceroides* and those of the recent *P. acerifolia* belong to the same basic lot. The later species has to be looked on as a relict and not as a hybrid of unknown origin.

Taxonomy and paleobotany do not belong to the fields in which the reviewer feels familiar. So the following critical remarks will be restricted to the biometrical methods, without taking into consideration the value of the article for the progress of knowledge in other fields.

If one takes the standpoint that, compared with a significant and interesting result, the elegance of the methods by which it was reached is without importance, then the discussion is finished. When, on the other hand, the opinion is stated, that just for the introduction of new methods (biometry) into an established field of research (taxonomic paleobotany) the use of the most modern level of these methods should be considered from the beginning, then the paper presented to us is worth a critical evaluation.

The insight, that quantitative methods with biometric analysis of the data should be used when simple morphological comparisons do not solve the question, is familiar to experimental biologists, but in the field discussed here it has not been widely distributed till now. So we have first to acknowledge the efforts of the author in making measurements and calculations. But a thorough study of the paper leaves the impression of a discrepancy between the very good statements about the path which should be followed and the methods used in doing so. Viewed from the modern standing of biometry, the methods used in this paper are rather primitive. The author combines in an unusual manner a complete understanding of the way of argumentation and the aims of quantitative biology with an inadequate knowledge of how to use the tools. Such a criticism needs an explanation.

Let us first look for the positive aspects of the research presented to the reader. The form of the leaves is an important criterium for the taxonomy of the genus *Platanus*. The fossil material consists of a population of leaves, for which the relations to a certain individuum or a defined developmental stage are unknown. It is rightly appreciated that the goal cannot be to compare single fossil leaves with leaves of recent plants, but that the fossil population as a whole has to be put into the framework of recent populations. The knowledge of the variability of characters between recent populations is therefore of greater importance for the position which will be given to the fossils than the analysis of the sources of variation within the populations, even if these ought to be taken into consideration for a quantitative description of some characters in the taxonomy of recent plants. As a consequence, they are neglected in the investigation of the recent forms presented here. From each of two populations of plane-trees a random sample of leaves is taken. By this method of sampling, the relation of the sample to the whole population from which it is taken is the same for both the fossil and the recent material.

The form of leaves in a population can be described by a multitude of quantitative data. To find the variability of the form, two characters of taxonomic value were chosen, the number of lobes and the form of the leaf-basis. The distributions are given in two tables, one for each population. The number of leaves investigated is included somewhere in the text, in the tables only percentages are found. A biologist with training in biometry would have made only one table with the original numbers and a chi-square-test for the homogeneity of the distributions. The criteria used in these tables cannot be seen in the fossil material. So the problem was to find a character in the recent leaves that could be measured on the fossils as well. In following this line, the author proves that the angle formed by the midrib and the main side-nerves at the basis of the leaf as one character, and the form of the leaves, described by the length-width-ratio, as the other character, are correlated in such a way, that this angle is sufficient to describe the form of the leaf and can be used as a taxonomic criterium. A table with some data to illustrate this statement is included in the text. It would surely have been possible to give a complete correlation-table of all the measurements, using nearly the same amount of space. This would have given the basis for a description of the relations between all the characters included in the investigation by means of a modern multivariate analysis, and this would have given a much better basis for statements about the fossil species.

The angle mentioned above can be measured with sufficient accuracy even on fragments of fossil leaves. The distribution of the angles measured on samples of fossil and recent material is determined. But for the calculations of the means and standard-deviations, instead of the formulae which can be found in all textbooks on statistics and biometry of post-war origin, the author uses the prehistoric method of condensing the data into a very crude scheme of classes and working with the deviation from a provisionally accepted mean. And all the steps of the calculations are included in a large table! After that, the comparison of the parameters of the samples is performed with adequate tests. The results of these comparisons give the basis for the conclusions mentioned at the beginning. But why, when the existence of a normal distribution and homogeneity is proved, not use a simple analysis of variance? I hope the author will have the courage and support to continue the line of research he has started here, and at the same time develop enough self-criticism first to improve his knowledge of biometric methods under good guidance (in Hungary there are good biometricians). I am sure of his success.

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IS IT POSSIBLE THAT PLANT SPECIES FROM THE PRE-GLACIAL TERTIARY TIMES HAVE SURVIVED IN THE CARPATHIAN BASIN?

In connection with Ferenc Radics's paper on the origin of *Platanus acerifolia* two problems arise.

1. The hybrid origin *(Platanus occidentalis* and *orientalis)* is not likely, because the progenies of *Pl. acerifolia* cultivated in Hungary — when grown from seed — do not show the properties generally characterizing hybrids, that the progenies throw back — either totally or with intergrades — to the two parent species. This was observed at the Buda abutment of the Elisabeth-bridge blown up during the second world war, where from the fruits of the two *Pl. acerifolia* trees of the Rudas-baths hundreds of platan seedlings emerged on the ruinous Danube embankment, whose leaves were perfectly identical with those of the parent trees.

2. Could plant species from the Tertiary before the ice-age survive in the Carpathian basin too? There are very few plant species in Hungary whose continuity from the Tertiary have been pointed out by the Hungarian literature. A great many plants of the Tertiary times are only thought to have gradually returned to Hungary in the post-glacial times. Information on the changes in the Hungarian flora of the ice-age is given first of all by the pollen analysis data, and as for the distribution of the Tertiary species no such data are available. It is impossible that they should exist at that, because the pollen analysis can only show the distribution of air pollinated species, so the insect pollinated species, like *Rosaceae*, *Tiliaceae*, etc., are completely absent. But here we must take it into consideration that the Carpathian basin was not totally covered with ice or snow during the glacial period, only the ice-flows of the higher mountains descended, as shown by the boundary lines of the moraines left behind. In the ice-free corners and valleys of the mountains and hills many a plant species may have survived the ice-age, in the first place stoloniferous trees, shrubs and herbaceous plants. E. g. of the arboraceous plants *Sorbus domestica* from the Sarmatian — its leaf imprint has been found by Lake Balaton — is mentioned by Andreánszky as *S. aria* cfr. *aucuparia*, but Z. Kárpáti and myself regard it as typical *S. domestica*. This species is even today often encountered in the Hungarian forests. A trunk of *Populus tremula* (its fossil name is *Populoxylon*) from the Sarmatian age is known at Mikófalva. *Carpinus neilreichii*, Kovács's find of the Sarmatian age, corresponds to *Carpinus orientalis* which was discovered several decades ago by Ádám Boros in the Vértes Mountains around Csákvár and found later at Alcsut and other places too. *Castanea sativa* which occurs in the Sarmatian fossils is existing today at a number of ancient growing sites in Hungary. *Cotinus coggygria* Scop. occurred in the Sarmatian, its fossil name is *Rhus palaecotinus;* it now lives in Hungary in a particularly great diversity in the Vértes Mountains. The leaf of *Lonicera caprifolium* is similar to the Sarmatian andesite tuff fossil of *Lonicera Lipthayana* at Nógrádszakáll. It is a species of eastern sub-mediterranean distribution extending to Kurdistan; in Hungary the literature considers it to be a native species in the Vértes and in some places of Transdanubia, but the North-Hungarian specimens are generally regarded as garden varieties grown wild. However, in an ancient northern oak forest of Sárhegy, near Gyöngyös, in 1972 we found with József Újhelyi an about 18—20 m stolon which makes it unlikely that it is a feral plant, as no cultivated form of the species was found in the neighbourhood.

Herbaceous plants: *Vicia sparsiflora*, a species of the Balkan and South-Italy, occurs in several places of Hungary, in forests with limestone and loess, e.g. in those of the Pilis and Buda Mountains. Soó himself regards it as a relict species. *Symphytum bohemicum* Schmidt, the yellow flowered comfrey, is frequently encountered in the valleys of the Mátra; from here it has descended to the moist meadows of the Great Hungarian Plain. It is also an ancient relict species in connection with which it should be taken in consideration that not only the rocky mountain sides, corners and canyons but smaller streamlets too were suitable for ancient plant species to survive in. The common reed which tolerates the died out water sides better than the permanent floods is a cosmopolitan species; in Hungary it is known from the Sarmatian and Levantine times too under the fossil name *Phragmites oeningensis A. Br.*

Helleborus odorus may be mentioned as an example of permanent shading. It is an eastern Alpine, Appenine, Balkan and South-Transdanubian species. Around 1930 I planted a specimen in the shade of the six storey building of Móricz Zsigmond Circus No. 3, Budapest, which up to the present day (1974) has blossomed but once — in 1968 —, and during the whole period only developed a single leaf a year. The place receives direct sunshine only in the summer half-year for one to three hours a day, and even that is reduced by the shadow of lilac shrubs, maple- and locust-trees.

After all, Radics's opinion that the *Platanus acerifolia* living today are identical with the Tertiary fossil *Platanus aceroides* (Goepp) Heer can be considered correct.

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APART FROM THE ANGLE OF DEVIATION OF THE LATERAL RIB SHOULD OTHER CHARACTERISTICS OF THE LEAVES OF PLATANUS ACERIFOLIA (AIT.) WILLD. AND PLATANUS ACEROIDES (GOEPP.) HEER BE COMPARED?

It is a great honour for me to contribute to your section "Forum". However, it is with mixed feelings that I write this review because I am unfamilar with the conditions under which the work has been carried out; some of my criticisms may thus appear somewhat pompous and unfair but this is not intended.

The author has presented his paper in English, this is praiseworthy and very convenient for the international scientific community. However, the English is by no means perfect and a few editorial corrections would have made the work much more readable. For example, *Platanus* is called the plane or plane-tree in English and not the frequently adopted word "platan", the plural of taxon is taxa (not taxons) but mostly it would have been better to replace taxon with the word species, on several occasions "of" and "after" would be better replaced with "for" or "from", and numerous other small mistakes. Fortunately, the meaning of the text is not seriously obscured by grammatical errors.

Still considering editorial matters, there are signs of carelessness. For example, the references on page 232 are particularly bad. In the first reference the page numbers are missing. The second reference cited as: "AITON, W. TOWN (1813): Hort. Kew. London, II, 1—368" refers to Aiton, W. Hortus Kewensis, edition 2 by AITON, W. T. (1813): volume 5. Lower in the list "BROTERUS, F. A. (1804): Flora Lusitania" should be written: BROTERO, F. DI A. (1804): Flora Lusitanica: — "CAVALLI, L.—SFORZA" should be written CAVALLI-SFORZA, L. On the first occurrence "FISCHER, R. A.—JATES, FR." should be written FISHER, R. A. and YATES, F. ("EDINBOURGH" should be Edinburgh and I am not quite sure what "I—X+ +I—138" following the reference means). The second appearance of "FISCHER—YATES" is a real howler and should read IORDANOV, D. *et al.* and "Blagaria" should be Bulgariya. There are also additional mistakes in some other references. Up to now my criticisms have been aimed at more or less simple editorial matters which in some countries are, at least, partly the responsibility of the editor of the journal. However, this obvious carelessness forces the reviewer to really put this work under the lens.

Following the International Code of Botanical Nomenclature the correct name for *Platanus acerifolia* (Ait.) Willd, is probably *Platanus hybrida* Brotero, Fl. Lusit. 2: 487 (1804). Brotero's work is earlier than Willdenow's and on page 487 of Flora Lusitanica, Brotero clearly describes this plant at the rank of species. In his notes below the diagnosis he writes: "ut videtur, hybrida Plat. — *orientalis* et Plat. *occidentalis* proles" — as it seems, a hybrid offspring of *P. orientalis* and *P. occidentalis.* The hybrid origin is no more than an opinion and does not affect the nomenclature neither does the later opinion in the same notes that it may also be a variety of *P. orientalis.* Brotero originally called it *Platanus hybridus* but all trees ending in "us" are considered to be female.

The origins of *Platanus hybrida* are still open to discussion. At the present time, at least, it has not been found as a 'wild' species in a more or less natal habitat. It is variously interpreted as a race of the Balkan endemic species *P. orientalis* or a hybrid between *P. orientalis* and the North American species *P. occidentalis.* The status of *P. hybrida* is discussed in detail by Li, H.-L. (1957): The origin and history of the cultivated plane-trees. Morris Arb. Bull. 8: 3—9, 26—31. Unfortunately, this work by Li was apparently not seen by Dr. F. Radics or else he would have probably modified his approach to this problem. The most important observation summarized in this work is that *P. hybrida* is extremely variable, and, at least, eight cultivars are recognized, six of which were once recognized as distinct species. Much of this information is also available in Rehder, A. (1940): Manual of cultivated trees and shrubs ed. 2, New York, and in other horticultural books. Although *P. hybrida* is usually propagated by stem cuttings, some races are fertile and are known to yield variable offspring. *P. occidentalis* is also known to be genetically variable and *P. orientalis* also shows considerable variation in leaf-shape.

Given the information that *P. hybrida* is genetically variable it seems to me wrong to choose material from relatively uniform trees from the Budapest Town Park and an alley at Gizella-telep, Visegrád as the standard representatives of present-day *P. hybrida.* It would surely have been better to get leaves from many different gardens and to have checked herbarium material from foreign institutes.

However, a more serious objection is that the other eight or nine present-day species of *Platanus* were not really thoroughly examined. A superficial comparison was made with fossil leaves of *P. acerifolia* and some illustrations of *P. orientalis* and *P. occidentalis* and 15 leaves of *P. occidentalis* (origin not stated) and were found to be different; both of these species are variable in leaf-shape and this was also not taken into consideration.

Another feature of this paper that makes me somewhat uneasy is the reliance on essentially a single character: the angle of deviation of the lateral rib. The leaves of flowering plants have many characteristics which have been proved useful in taxonomic delimitation. This is well reviewed by Dilcher, D. L. (1974): Approaches to the identification of Angiosperm leaf remains. Bot. Rev. 40 (1): 1—157. If all these other characters are of no diagnostic value in the taxonomy of *Platanus* then the author should have, at least, pointed it out. That the angle of deviation of the lateral ribs in the leaves of fossil and present-day material of *P. hybrid* is the same is not being disputed. The title of this paper claims no more but the text of the paper makes further claims.

Personally, I have never been really happy with the conception of *P. hybrida* as a hybrid or a race of an existing species. To use a negative argument, Dr. F. Radics has not convinced me that the fossil species *P. acerifolia* and the present-day species *P. hybrida* are not the same but, at the same time he has not convinced me that they are the same. Before I will be convinced that *Platanus hybrida* was present during the Tertiary Period in Europe I would like more evidence from more characters and a comparison with much more material from present-day species.

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IS THE VARIATION OF VEIN ANGLES SUFFICIENT TO ESTABLISH THE GENETIC RELATION BETWEEN PLATANUS ACEROIDES AND P. ACERIFOLIA?

On the basis of the reasons given in his paper the author arrives at the conclusion: "The statistical comparison of the leaf parameters of the Tertiary (Sarmatian) platan *P. aceroides* and the recent *Platanus acerifolia* reveals a great likeness of the two plane-trees. Accordingly, and on the basis of supplementary investigations *P. acerifolia* can be supposed to be neither a hybrid of the eastern *(P. orientalis)* and western *(P. occidentalis)* platan, nor the progeny of one or the other, but a type of the undifferentiated Tertiary "primaeval platan". To prove this statement he made a statistical comparison between them.

Thus, by the above conclusion the author wishes to answer two fundamental questions. One of them mainly concerns the origin of *P. acerifolia*, the other attempts to bring Heer's *P. aceroides*, the Tertiary primaeval platan into connection with the recent *P. acerifolia*.

With the author's first point, namely, that *P. acerifolia* is no hybrid of the eastern and western platan we more or less agree. Although both in Hungary and in the relevant literature in general *P. acerifolia* is thought to be nothing else than a hybrid of *P. occidentalis* and *P. orientalis* (Krüssmann). Yet, why is this supposition improbable?

First of all, there is no positive evidence of such a hybridization between the two species having ever or anywhere taken place. Such a recent and supposed hybridization is inconsistent with the considerably distant, disjunctive distribution of the two typical species, too. The geographic distribution areas of *P. occidentalis* — together with a number of other platan species, e. g. *P. Wrightii*, *P. racemosa*, *P. chicepensis* — are the southern part of the United States, Mexico and California. On the other hand, the distribution areas of *P. orientalis* the other supposed parent, — together with *P. acerifolia*, *P. digitata* and *P. orientalior* — are Southern Europe, Greece and Asia Minor. These two continents are so far away from one another that after the Tertiary, hybridization between them could only have taken place after the discovery of America at the most. As, however, nothing is known about this, it is not likely at all.

But in connection with a hybridization like that another genetic problem, too, has to be taken into consideration. Namely, when such a crossing takes place — should it be either an intermediary or a dominant inheritance — according to Mendel's segregation rules the properties of the original two homozygous parents ought to appear in some form in the progenies. And in *P. acerifolia* such phenomena have not been observed so far. Namely, *P. acerifolia* has always typical *acerifolia* progenies, though it is true that slightly different forms of *P. acerifolia* are now known these features are, however, quite new and did not exist in the original parents.

And if *P. acerifolia* really were a hybrid, then this phenomenon could be pointed out by biometrical examinations of the pollens. Namely, in case the biometric examination of the pollen of the monoecious *P. acerifolia* showed a two-peak curve, then this really would prove its hybrid nature, while a single-peak curve would contradict it. (To my best knowledge, such investigations have not been made so far.)

The views about the hybrid nature of *P. acerifolia* are not adopted by other platan researchers either; according to Ferguson e. g. hybridization between *P. orientalis* and *P. occidentalis* is not likely. In this point we thus agree with the author's conclusion that *P. acerifolia* is not a hybrid of *P. occidentalis* and *P. orientalis.*

The answer to the author's other problem is not as simple as that, and our opinion somewhat differs from his. Since Heer's fossil *P. aceroides* shows a great likeness with the recent *P. orientalis* and *acerifolia*, respectively, concerning the parameters of the vein angles, in the author's opinion there may be a genetic relation between them. Therefore it was not for nothing that the idea of *P. acerifolia* being a variation of *P. orientalis* arose. And there is some truth in it, at that. As a common feature of the two, the bark comes off the trunk in large sheets. There is some difference between them, however, in the size of the leaves and in the parameters of the vein angles. Namely, the leaves of *P. acerifolia* are usually larger (12—25 cm), while the leaf size of *P. orientalis* ranges between 10 and 20 cm. As a further difference, the fruits hang on the fruit stem in ones-threes in *P. acerifolia*, and in twos-sixes in *P. orientalis.* (Krüssmann). Thus, in this respect *P. orientalis* shows a greater similarity to *P. levis*, a species from the Bohemian cretaceous period.

The only thing left is to examine the leaf vein angles characteristic of the plane-trees. According to the original data the leaf vein angle of Heer's *P. aceroides* ranges from 24 to 48°. (In the drawing presented by him it is only 32°.) On the other hand, the leaf vein angle of *P. acerifolia* studied by the author was 23—52°, but according to our own investigations made on *P. acerifolia* at Szeged it may even reach 55—58°. Moreover, on the juvenal shoots of the plane-trees at Szeged intermediate and gradual forms can be observed, that is, from quite simple, unbroken, almost egg-shaped leaves, through slightly widening ones with initial lobes to those with 3—5 and even 7 lobes many forms can be found. The vein angle of unbroken egg-shaped leaves sometimes hardly reaches 30°. The author is right in saying that the morphology of plane-trees has not yet been fully elaborated and would deserve further investigations.

The author still supposes genetic relations between *P. acerifolia*, and *P. orientalis*, *P. occidentalis* and *P. aceroides*, mainly on the basis of the similarity of the vein angles. In our opinion, however, from the parameters of the vein angles, that is, on the basis of a single aspect, no far-reaching conclusions can be drawn. There may occur e.g. a case when a smaller leaf has a larger vein angle than a larger leaf of the same tree. A 25 cm wide and 17 cm long leaf of a plane-tree at Szeged had a 57° vein angle, while on a 28 cm wide and 21 cm long leaf of the same tree the vein angle was only 50°. Thus the supposition that larger leaves have larger vein angles — which gradually grew over the geological periods — is not of general validity. All this can, otherwise, be proved by placing a smaller leaf of *P. acerifolia* over a larger one in such a way that the two main ribs cover each other, when the lateral vein on the smaller leaf encloses a somewhat larger angle with the main rib than the first vein of the larger leaf.

Since the author thinks to have found similar correlations between the leaf vein angles of Heer's *P. aceroides* and the recent *P. acerifolia* he arrives at the conclusion that the latter two can be regarded as a sort of primaeval platan, and that *P. occidentalis* and *P. orientalis* are such progenies. The same opinion is otherwise held by Gábor Andreánszky who wrote: "Wir sind demnach der Meinung dass *Pl. aceroides* Goepp., eine Urform beiden genannten Arten *(P. occidentalis* and *P. orientalis)* gewesen ist."

Andreánszky supposes thus that *P. aceroides* was a kind of primaeval plane-tree from which *P. occidentalis* and *P. orientalis* may have originated. According to the author *P. acerifolia* would be the same. We do not think, however, that *P. aceroides* is the very primaeval platan from which the former species have developed. In our opinion in the cretaceous period the primaeval platan had not yet leaves with 3 or 5 lobes like the Sarmatian *P. aceroides;* its leaves must have been simple, whole egg-shaped leaves similar to those found today on the juvenal shoots, and became wider and lobed only later. This stage probably set in during the cretaceous and not in the Sarmatian period, when it spread all over the world. (By the way, the author speaks of whole leaves, though Heer's platan leaf which he considers to be from a primaeval plane-tree is not whole but has three distinct lobes; see the drawing). (Gothan Weyland: Lehrbuch der Paläobotanik. 399 p.)

Anyway, when discussing such an important problem all platan leaves, or at least those found in Europe should be included in the comparison, as from different parts of the Globe some 40 platan fossils are known. In Kristofovics's important monograph about four types of platan leaves are presented.

In our opinion the Tertiary *P. aceroides* shows a more developed though intermediate state, while species of the most developed form are represented as relicts by an 50 m high and 5 m diameter *P. occidentalis* in North-America, a 40 m high and 4 m diameter and 13 m girth *P. orientalis* in the Botanical Garden of Trsteno, Yugoslavia, and a 40 m high and 2.25 m girth *P. acerifolia* at Visegrád (Hungary).

Since the variation of vein angles in itself is not considered to be sufficient to prove the genetic relation of *P. aceroides* and *P. acerifolia*, it is more likely that *P. acerifolia* has survived as a living relict since the Sarmatian up to the present day neither as a progeny of *P. aceriodes* nor as a hybrid of *P. orientalis* and *P. occidentalis*, but together with them.

We agree, on the other hand, with the author and other platan researchers that many morphological, anatomical and palinological investigations will have to be made until the phylogenesis of the plane-trees will be satisfactorily clarified. In any case, the problem is interesting and worth being dealt with.

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WHAT WERE THE TAXONOMICAL DEVELOPMENT AND CHANGES OF THE CONTENTS OF THE LATE-TERTIARY MIDDLE EUROPEAN PLANE-TREE LEAVES?

The presented manuscript is a statistic-comparative work of the modern species *Platanus acerifolia* and the fossil species *Platanus aceroides.* Its introduction represents a very brief, but quite necessary discussion of the historical development of the conceptions of the modern species: mostly it is considered as a hybrid of the occidental and oriental species. This suspicion is usually accepted by means of the geographical position of the occurrence of *Platanus acerifolia* and its presence in a horticulture exclusively (!)

Quite briefly the author deals with the oldest finds of *Platanus aceroides* in Tertiary floras only. I am afraid the author was too much concentrated on the comparison of Hungarian fossil specimens with the results of the measuring of the modern material, so that he omitted the nomenclatorical as well as the taxonomical development and changes of the contents of the Late-Tertiary Middle European plane-tree leaves. See below!

The author had mostly only fragmentary leaf-remnants and therefore he concentrated his attention on the investigation of the branching of the main ribs and of the leaf-base. From the statistical and methodical point of view it seems, that the number of investigated modern leaves is sufficient. Very interesting and suggestive is an excursion into antique and more later historical indications of living species in Europe.

Though I did not see the plates I suppose the plates prove properly demonstrable evidences of the results of the statistic observations.

Although it seems that a lot of characteristics testify the taxonomical proximity of these fossil and modern plane-trees, I am of the opinion that it is not quite correct to identify both species together, for the insufficient knowledge of the biological essentiality of "extinct" species *Platanus aceroides*. But, if the author is of the opinion his arguments are weighty and convincing enough, he will have to consider (for nomenclatorical reasons) the fossil (though younger) species *P. aceroides* for the variety of living species of *P. acerifolia:* e.g.: *Platanus acerifolia* var. *acerifolia* (as the typical one) and fossil *Platanus acerifolia* var. *aceroides* (resp. more correctly: *Platanus acerifolia* var. *platanifolia*).

From the Moravian Neogene there are many well known finds of *Platanus aceroides:* I recommend compiling the monograph published by KNOBLOCH 1969: Tertiäre Floren von Mähren. Moravian Museum, Brno; in this paper the author can find a lot of indications of the occurrence of the fossil plane-tree (discussed species), incl. valid nomenclatorical changes. The next remark is in: SITÁR 1973: Die fossile Flora Sarmatischer Sedimente aus der Umgebung von Mociar etc. — Acta Geol. et Geogr. Univers. Comenianae, ser. Geologica, Nr. 26, Bratislava. Additionally: SVAREVA 1969: (Paleont. sbornik; AN USSR) Mioc. flora Myschina v Predkarpatii; KOLAKOVSKIJ 1958: Flora Meore Atava; KUTUZKINA 1958: Platan iz Sarmata. — Bot. zhurn., 13, USSR; SAFAROV 1967: In: Biull. Mosk. Obsch. Ispit. Prirody, Biol., 77, 3, USSR atd. All these enumerated papers deal with very interesting finds of Miocene *Platanus* remnants, mostly described as *Platanus orientalis* related forms.

I hope all these remarks can help the author essentially. Nowadays it is clear, that the development of the genus *Platanus* during the Tertiary had taken place in two directions (in Europe): the first branch in the direction of the simple-leaf species *(P. neptunii, P. keeri)* under the influence of hygromeso (mega) therm climatic conditions (with a relic in SE Asia), the second one (in the region of temperate climate) developed lobate leaves and, probably, led to the type *P. acerifolia* (?).

Radics's contribution to the problem of genus *Platanus* is very valuable, interesting and it is to be recommended for printing in every case.

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IS THE VARIABILITY OF PLATANUS ACERIFOLIA (AIT.) WILLD. POPULATIONS CAUSED BY THE PROPAGATION METHODS EMPLOYED UNDER CULTIVATION?

A. Rehder (Manual of Cultivated Trees and Shrubs 1940) also considers *Platanus acerifolia* (Ait.) Willd. as an uncertain hybrid of *P. occidentalis* × *orientalis* and dates its production, or rather its appearance under cultivation, from before 1700. He also stresses the variable formation of the leaf form, principally the leaf base.

The widely cultivated planes in Hungary, which are without doubt *P. acerifolia*, are exceptionally diverse. This is exhibited primarily in the shape and lobular nature of the leaves and in the different development of the bracteate leaves, which are often very large. The number of flower-heads varies from 1—6, while the flowers themselves are equally variable, being 4—6-membered. The pubescence found between the fruits in *P. orientalis* and absent in *P. occidentalis* are sometimes present in *P. acerifolia* and sometimes absent. This all points to hybrid origin.

The propagation methods employed under cultivation also play a part in the variability of the planes. Some of the saplings are produced from seed, i.e. the population would exhibit variability even in the case of homozygotes. At the same time, many tree nurseries propagate the most attractive individuals by taking cuttings, i.e. they produce homogeneous clones. By the time the saplings reach their final destination, however, they have usually been mixed up, so it is very rare to see uniform stands in an avenue of trees.

The identification of *P. acerifolia* with the ancestral plane is in fact logical from a genetic point of view, since its hereditary material doubtless existed earlier in *P. aceroides*. But this also applies, of course, to *P. occidentalis* and *orientalis*, whose morphological characters recall in a similar manner the aceroides ancestor. Thus we can also accept the conclusions of K. Kramer (Fossile Pflanzen aus der Braunkohlenzeit, in Mitt. Deutsche Dendrol. Ges. 67, 199—233, 1974) who identifies the common fossil plane leaves with the living *P. occidentalis*. Kramer also draws attention to the danger attached to comparing the fragmentary material only on a morphological basis. As an example of this he gives illustrations of the convergent leaf forms of the *Aceracae*, *Hamamelidacae*, *Araliacae*, *Platanacae*, etc. It may perhaps be useful to report Kramer's concentrated summary of his views on the identification and nomenclature of the fossil and living forms in the original:

"Abschliessend sei deshalb noch darauf hingewiesen, dass es sich um Vergleichsarten, nicht um den direkten Nachweis dieser Arten im Tertier handelt. Was wir als Fossil in Händen haben und vergleichen können, ist stets nur ein Teil, ein Fragmentum (Blatt, Frucht, Same, Holz usw.) nie die ganze Pflanze mit all ihren morphologischen, anatomischen und physiologischen Merkmalen und Eigenschaften. Allein die Kenntnisse dieses Totalen aber würde den Nachweis der wirklichen Übereinstimmung mit einer rezenten Art ermöglichen und seine Identität mit ihr bezeugen. Das ist auch der Grund, weshalb selbst bei vollkommener Gleichheit eines fossilen Pflanzenorgans (z.B. eines Blattes) mit dem einer rezenten Art das Fossil nicht mit dem Namen der Vergleichsart belegt werden darf, sondern einen eigenen erhalten muss."

(Finally I must emphasise once again, that we are dealing with comparative species, not with the direct proof of these species in the Tertiary. The fossils available for comparison purposes are always only parts, fragments (leaf, fruit, seed, wood, etc.), never the whole plant with all its morphological, anatomical and physiological features and characteristics. But only the knowledge of this whole would make the proof of a genuine agreement with a living form possible and give witness to their common identity. This is also the reason why even when complete similarity exists between a fossil plant organ (e.g. a leaf) and that of a living form, the fossil may not be given the name of the comparative species, but must receive a separate name.)

In conclusion: through his breeding-domesticating activities man has undoubtedly accelerated the transformation, if you like evolution, of that living material which comes within his sphere of interest. This evolution often exhibits identical forms of organisation collaterally in various families. It would be of benefit if this parallelism were also to find expression in taxonomy, which, unfortunately, still restricts itself almost exclusively to morphological methods.

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HAS ANYTHING REALLY BEEN PROVED?

For the following review the manuscript of the above paper was available to the reviewer (without Tables!).

This paper represents a first attempt at comparing the living variety *Platanus acerifolia* (Ait.) Willd, with the fossil variety *Platanus aceroides* (Goepp.) Heer on a biometrical basis. In this way both corresponding and diverse characters could be mathematically demonstrated for the two forms. The paper is illustrated with living *P. acerifolia* material from the Budapest City Park and from Visegrád (100—140 leaves) and from Lower and Upper Sarmatia (45 leaves in all).

In the opinion of the reviewer, papers of this nature should only be written in very special cases. A comparison between just one living variety taken from a very small area with just one fossil variety taken from a limited stratigraphic range can only produce very limited results, which do not always justify the work invested in them. Similarly the repetition of countless introductory remarks (in several papers on the same subject) is no small burden on the literature, especially when these remarks are not exhaustive (thus, in this paper the living variety is dealt with relatively extensively with respect to synonymics and definition, while due attention has not been devoted to the fossil variety). When the present paper is considered from this point of view, although it supplies a number of concrete data on the biometric comparison of certain fossil individuals with respect to a living variety (which is unlikely to originate from a natural biotope), it gives no answer to many of the questions which are to be settled in this connection. In this particular case, a variation statistical analysis (using mathematical methods where necessary) should first be made of the variability in the leaf morphology of all the living varieties *(Platanus acerifolia, P. occidentalis, P. orientalis)* which come under consideration for a comparison with the New Tertiary fossil *Platanus* remains. *Platanus* fossil remains from strata of various eras should, in this case, also be examined biometrically (in oak varieties, for instance, it has been shown that the leaf surface, within the same variety, changed during the Meiocene). It would also be interesting to know whether the meiocene plane leaves really belong to the same variety as the pleistocene leaves, as is implied in this article, since this seems unlikely on other grounds. Despite a certain amount of concrete progress achieved by the comparison of living and fossil plane leaves, it seems to the reviewer that a more complex elaboration of the subject would have been in order.

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PLATANUS ACERIFOLIA (AIT.) WILLD. OR PLATANUS HYBRIDA BROT.?

The paper wishes to contribute new aspects to the solution of a nearly two centuries old problem by calling attention to the taxonomic utilizability of vein angles as parameters of platan leaves. It would be desirable to include this character in the investigations of the other platan species too, as up to now the platan studies have laid emphasis upon the shape, edge and lobes of the leaf blade and the form of the leaf shoulder.

Every attempt made to the complex solution of problems so far studied from a single aspect — e.g. on the basis of external morphology — is welcome in botany too. Most of the present difficulties of taxonomy can only be overcome by the joint application of the methods of various sciences, with unanimous results of extensive investigations.

The paper in question offers new evidence concerning the origin and taxonomic place of the Mediterranean platans. It produces measurement data to support the hypothesis — set up earlier by others too and considered as the most probable one — that the plane-tree grown everywhere in Europe *(P. acerifolia = P. hybrida)* is not a hybrid of the North-American and Eastern Mediterranean platan species. The author's statement that *P. acerifolia*, most likely growing wild in Southern Europe, is nothing else than a progeny of the fossil Sarmatian Central- and Southern European *P. aceroides* — having survived under special conditions — seems to be highly probable.

As regards the name of *P. acerifolia* it seems to be necessary to note — as it is not clear from the text — that according to the rules of the nomenclature the name *P. hybrida* Brot. (1804) has a priority over the later combination of *P. acerifolia* (Ait.) Willd. (1805) even if the former is not the right name referring to the hybridization. The literature on the subject (HADFIELD, M. 1960: The mystery of the London Plane. — Gard. Chron. **148** p. 422, 443, 462) ought to have been included in the references.

Among the references themselves a number of data have to be corrected. E.g. AITON "1813, Hort. Kew." has to be replaced by: 1811, Hortus Kewensis; BROTERUS Flora "Lusitania" by: Lusitanica; DECANDOLLE Vol. "XVI." by Vol. XVI/2.; "LOUDON, I. C. 1854 *Arboretum Britannicum*, I—VIII. . ." by: LOUDON, J. C. 1838 *Arboretum et Fruticetum Britannicum*, IV.; and in the title of WILLDENOW's book "a Linné" has to be substituted for "linnei". It is not clear either what "North American Flora in Bot. Gard. 1908 . . ." in the list may be. Neither author nor editor are given; on the other hand, extensive flora works are not usually published in periodicals.

It is worth considering whether the fractional values of the measured angles are rightly published in the decimal scale (the text of the paper even contains such expressions as 24.75° or 37.52°). The unit *g* (400 degrees "grad") often used in the west might have been more reasonably employed in the paper.

Finally, just a few words about the terminology used in the paper. Scientific publications cannot be without the Latin or Hungarian terms. It is a generally known basic rule that in scientific papers the use of terms should be consistent, unmistakable, not differing from the established terminology. (It is a different case when an author publishes a polemic paper with the purpose of changing an established and — in his opinion — incorrect term.) In the paper in question there are several misleading, questionable or inconsistently used Hungarian terms. The term "sinus of the leaf margin" e.g. is not in use. It is a question whether we should speak of "main ribs". The opinion that there is only one main rib (that is why it is called "main" rib), and if it branches off then we speak of primary or secondary lateral veins seems to be acceptable. The terms fruit ball and fruit spike in Figs I/3 and I/4, respectively, are inconsistently used. The two are the same: ball or capitulum, but by no means spike.

The paper uses for the most part terms of leaf morphology, among others the easily misunderstood term "leaf base". This requires some explanation since in the Hungarian literature the term "leaf base" is most regrettably used to designate two different concepts. This homonymous name may then cause troubles which, however, should be avoided. (Similar confusion may exist in German or English texts too, though perhaps in a lesser degree.) The term leaf base is used, on the one hand, to designate the lowermost of the three parts (leaf base, petiole and leaf blade) of the leaf *(fundus vel basis folii)*, while on the other hand it means the lowest part of the leaf blade *(fundus vel basis laminae folii)*. In the first case it means the lowermost part of the whole leaf or of the petiole *(basis petioli)*, respectively, that is the place where the leaf joins the node. This leaf base, though sometimes less differentiated, often may be modified e.g. into the well known leaf sheath *(vagina folii, Gramineae, Cyperaceae, Allium* spp., *Umbelliferae)*, or may be conspicously swollen (e.g. *Aesculus, Ailanthus*), or — as in the very case of platan (though the paper does not mention it) — have a peculiar conic shape, etc. In the other case, when only the lower part of the leaf blade *(basis laminae)* is meant by the term leaf base, the latter had better be replaced in Hungarian by the long since known and well proved, unmistakable term: "leaf shoulder". While the base of the petiole is little varied, the shoulder of the leaf may have highly diversified forms. The paper too describes some of them, and the figures 1—4 of Plate II show in a morphologically correct way leaves with obtuse, wedge-shaped, cut and sinuate-cordata shoulders. For the latter the term subcordatus is in itself incorrect. Down-bowing is not identical with subcordatus. The latter means an inverted heart shape while the leaf shoulder in question is sinuate-cordate.

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HAS THE CASE FOR THE DISTINCTNESS OF P. ACERIFOLIA REALLY BEEN PROVED?

The author proceeds from the well-known fact that the leaf structure of the fossil form *Platanus aceroides* (Goeff.) Heer corresponds almost perfectly with that of the living form *Platanus acerifolia* (Ait.) Willd. By means of a statistical analysis of the nervation angles, he attempts to prove that *Pl. acerifolia* is not, as is often assumed today, a cross between Pl. occidentalis and *Pl. orientalis*, but a distinct species originating from *Pl. aceroides.*

The question immediately arises, why this result has not been supported by a cytological, primarily a chromosome morphological investigation of the 3 living taxa mentioned; since a cytological examination of *Pl. acerifolia* for the hybrid nature in question would have produced definitive results and would have been no indirect conclusion.

It is also strange that, on the subject of the introduction of *Pl. acerifolia* into England, only the old works by Aiton (1789, 1813) are quoted, and not the study by Hadfield, M. (The mystery of the London Plane. — Gard. Chron., 148: 422, 443, 462. 1960), in which the supposed natural habitat of *Pl. acerifolia* in the Levant is questioned.

Merit is certainly due to the author for having statistically recorded the exact angle relationships in the nervation of the fossil species. But in our opinion this cannot be said for *Pl. acerifolia*, since only planes from two areas of cultivation have been evaluated, despite the fact that for *Pl. acerifolia* many varieties, which differ precisely in the leaf structure and nervation, are well-known and form an almost continuous series from *Pl. occidentalis* to *Pl. orientalis.* Thus in our opinion no leaf morphological proof for the distinctness of *Pl. acerifolia* has been put forward.

Had cytological facts been taken as the starting point, the present work would, in the case of a positive proof of the distinctness of *Pl. acerifolia*, have represented an excellent confirmation and supplementation. In any case, the question has been reopened to discussion.

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ARE THE FOSSIL PLATANUS LEAF REMAINS EQUIVALENT TO LEAVES OF RECENT PLATANS ORIGINATING FROM SINGLE BIOTOPES?

Radics's comparative study on platan leaves is of considerable importance from the point of view of objective, method and conclusions alike. Namely, in the biological literature of Hungary evolutionary studies directly utilizing the results of paleontology in ascertaining the actual course of phylogenesis are regrettably small in number. The author deserves credit for approaching the present by proceeding from the past, relying equally on the fossil documents of palaeobotany and the recent material of investigation. Beyond the choice of an evolutionary subject the theme itself is interesting too, partly because the taxonomic problems concerning *P. acerifolia* are unsolved even today, partly because in the extremely rich Sarmatian flora of Hungary several specimens of *P. aceroides* leaf have been found. It was not by chance that KNOBLOCH (1972, Földt, Közl. 102, p. 255.) emphasized the necessity of processing the Hungarian fossil platan material.

The solid quantitative basis of comparison provided by the data of angles enclosed by the main rib and the lateral leaf veins is remarkable from a methodological point of view. As to the sample taking, however, we have to note that it is not quite equivalent in the case of fossil and recent materials. The recent material of investigation is chosen from leaves of a given population of the same biotope fallen at a given time. In selecting the documents of the past the situation is — naturally — quite different. The material of the Sarmatian sites may originate from various florae of a time interval of 4—6 million years. During that period the prevailing topographic and climatic conditions of a region changed to a great extent. Isochronism might be spoken of in the case of leaf remains collected from the same bedding plane, but the evidence of their belonging to the same population would even then be missing.

Finally, as for the results, the author is right in pointing out that the relationship of *P. aceroides* and *P. acerifolia* suggested by the morphology of the leaf should be regarded as a work hypothesis. Unfortunately the leaf construction is not the most typical character of the *Angiospermae*, and in the case of fossil remains the possibility of similar forms and a high variability must be reckoned with. The material of palaeobotany is only suitable for approaching once living florae. On the other hand, the author deserves credit for exploiting by far the possibilities provided by the approach.

Potential progression is an essential element of any scientific work. In the present case it would be worth re-examining the fossil platan leaf material of the surrounding areas taking into consideration the older (Oligocene, Lower and Middle Miocene) and younger (Pliocene) occurrences. Knobloch's activity is noteworthy if only for the clarification of the questions

of nomenclature. As for the possible survival of *P. aceroides*, besides the Bulgarian find cited by the author it may be supported by the Czechoslovakian Pliocene data too.

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WHY IS P. ACERIFOLIA NOT TO BE FOUND ANYWHERE IN A WILD STATE?

Platanus acerifolia (Ait.) Willd. is probably the most commonly planted large tree in British cities and towns, especially in the capital — London which has given rise to the generally accepted English name of 'London Plane'.

Although it is now a widely planted tree throughout Europe, its origin has long been a source of debate and not infrequent argument. As far as I am aware *P. acerifolia* does not occur anywhere in a wild state and is only known from cultivation.

Two explanations are generally offered as to the origin of this plane. The first of these suggests that it arose as a sport, a form of the 'Oriental Plane' — *P. orientalis* L. The second and most commonly accepted theory is that it arose as a result of a cross between *P. orientalis* L. and the American *P. occidentalis* L.

Now F. Radics in the present paper puts forward a third theory which, based on statistical evidence, suggests that *P. acerifolia* is a relict species derived from the fossil species *P. aceriodes* (Geopp.) Heer.

Radics theory is certainly interesting and novel and might well repay further study. However, as it presently stands, it leaves too many unanswered questions and does not entirely nor satisfactorily dispose of the original theories as mentioned above.

All living species of *Platanus*, with the exception of a doubtful species from S. E. Asia, are essentially very much alike, and the numerous fossil planes are said to resemble them fairly closely. The living species are distinguished from each other by leaf-shape, leaf-indumentum and fruits. Surely it is impossible to make a valid comparison between a living and a fossil plant unless all the relevant taxonomic characters are preserved in the fossil — and clearly they are not in the imprints studied by the author.

The only character relied upon by the author is the angles subtended by the main veins, but before deciding whether this is a valid taxonomic character it is necessary to study in detail all the living species in order to ascertain whether they can be distinguished by the character. It simply is not good enough merely to assume that a character such as this is of paramount importance merely because it happens to be the only one available for study.

Another question which Radics paper fails to answer satisfactorily is why *P. acerifolia* is not to be found anywhere in a wild state. This is especially puzzling when one considers that several other supposed Tertiary relicts such as *Parrotia persica*, *Gleditsia caspica* and *Platanus orientalis* itself still survive in a wild state.

On the same theme, why is it that such an outstanding tree as this should not be noticed and recognized as different until the late 17th century?

The author does not sufficiently explain the variability of *P. acerifolia*. There are at least four named forms in cultivation. No mention is made of the name *P. hispanica* Muenchh. which would appear to be the earliest valid name for this tree, whilst the author's List of

References is very incomplete, not even mentioning Professor A. Henry's paper which, whilst debatable, is possibly the most important contribution to the subject.

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PLATANUS ACERIFOLIA (AIT.) WILLD. OR PLATANUS ORIENTALIS VAR. ACERIFOLIA AIT.?

As to its frequency among the old trees of the Hungarian settlements and alleys platan is, in fact, in the third place (on the average of 23 towns). Owing to the decreasing tendency of road construction and plantation (for known reasons) the number of platans diminishes. The platans of Margaret Island (Budapest), Esztergom, Eger and of several historical parks (Acsád, Martonvásár, Vácrátót (Arboretum), etc.) are famous.

Of the wild platan species *Platanus orientalis* L. in southern Bulgaria and Macedonia (Yugoslavia) is nearest to Hungary. It is interesting that it grew wild in Hungary only in a very low number compared e.g. to masses of *Ailanthus altissima*, *Amorpha fruticosa*, *Acer negundo*, etc. that ran wild. According to the nursery data (Probocskay) it is easy to propagate both from seed (through it has many empty seeds) and in a vegetative way (cuttings, layers).

Fossil platan occurrence is known from the cretaceous period. In the Tertiary the platan was already distributed almost everywhere in the northern hemisphere.

The variability of the Platanus genus. Today the platan species occur on a relatively small area, and the authors usually acknowledge the following ones:

American species	*Eurasian species*
P. occidentalis L.	*P. orientalis* L.
P. racemosa Nutt.	*P. acerifolia* (Ait.) Willd.
P. wrightii S. Wats.	

In our opinion, although the family *Platanaceae* itself has become extremely isolated, a considerable variability can be observed on the area of the mentioned species and in their plantation zones, respectively. According to Andreánszky (1959) the Hungarian fossil platan data also give evidence of a great variability.

When Radics in his paper published in No 24/1—2 of Acta Agronomica disputed the hybrid origin of *P. acerifolia*, and identified this recent species with the fossil *P. aceroides* (Goepp.) Heer built his "decisive" arguments, in fact, only on the leaves.

It should be noted here, that a number of authors acknowledge some 11 plant species (e.g. the *P. orientalis* character *P. hispanica*, *P. cuneata* and *P. digitata* are separate species in Sokolov's work; III. 1954). The relevant literature contains at least 24 — valid and invalid — names of species rank which refer to recent platans; besides them nearly 30 infraspecific taxons have been distinguished by various authors (see e.g. Rehder 1949).

Nomenclature relations of Platanus acerifolia. Data collected so far cannot be left out of consideration (see e.g. Krüssmann's work; II. 1962). Unfortunately the author did not take the tendencies found there into account. Although it must be noted that the nomenclature often conceals rather than expresses the phylogenetic aspects (e.g. in the case of the cherry).

Some authors (e.g. KRÜSSMANN l.c.) underline the hybrid character of *P. acerifolia* by indicating the synonym. But even those authors are not few in number who list *P. orientalis* var. *acerifolia* Ait. as a synonym of *P. acerifolia* (e.g. L. H. BAILEY, 4. ed., 1958; GRUBOV apud SOKOLOV l.c., BEISSNER, SCHELLE *et* ZABEL, 1902, etc.). In the opinion of others *P. acerifolia* is a cultivated form of *P. orientalis* (e.g. Soó 1966; PALAMAREV, Fl. R. P. Bulgaricae, 1970).

According to Radics Palamarev's statement that *P. aceroides* (Goepp.) Heer has been found not only in the Hungarian Pliocene but also in the Bulgarian Pleistocene is of great importance. It is a valuable statement for us too, because in Bulgaria (e.g. in the streets of Plovdiv) the so called typical *orientalis* and *acerifolia* trees occur in mixed plantation.

All these findings and names seem to confirm — more than anything — that the correct name of *P. acerifolia* is *P. orientalis* var. *acerifolia* Ait., and even if it means a transition to the *occidentalis*, on the basis of a number of morphogenetic characters is still closer to the *orientalis*.

Many authors have dealt with the "intermediate" species (in Hungary Kárpáti Z.) and introgressive hybridization. This would be a very lengthy subject to discuss here; but it must by all means be noted that it would not be surprising at all if wild stands of *acerifolia* were discovered, just as the occurrence of the similarly Tertiary origin *Aesculus hippocastanum* in the Balkan is not surprising any longer.

On the other hand, the fact that *Fagus moesiaca* and *Cornus hungarica* are not found on continuous areas but occur sporadically, in smaller populations — from Poland to the Balkan in various *Fagus silvatica* and *Cornus sanguinea* populations, respectively — is still under discussion.

Morphological, morphogenetical considerations. As mentioned before, Radics (naturally for objective reasons too) performed the statistical comparison of leaf data on the basis of only two planted *acerifolia* stands and *occidentalis* leaf drawings. So the data are not quite convincing, and are very few in number to support the theory. In spite of this, the conception is remarkable, and we think that it means only the beginning of a work.

The statement would be convincing if a detailed morphological comparison of at least those recent species that can be taken into account were carried out. E.g. the crown shape of *Platanus acerifolia* has so far been found identical with that of the *orientalis*, its fruit is also of very similar form with its tapering tip; the larger number of the fruit branches and the glabrate vein corners are also *orientalis* characters (Krüssmann). The height of the Hungarian cultivars is not considered to be a decisive character. (The question of how high a domestic tree species may grow can best be studied in the Bialowieza Reservatum, Poland; the alders *(Alnus glutinosa)* and the little-leaf lindens *(Tilia cordata)* generally are twice as high there as in Hungary.)

Conclusions. The author's statement that *P. acerifolia* is identical or closely related with the late Tertiary *P. aceroides* is considered to be acceptable. Similar examples of the late Tertiary occurrence of other Hungarian tree species can be found in large numbers (e.g. *Pyrus pyraster, Vitis silvestris, Alnus,* Juglans etc.).

The possibility of hybridization cannot be excluded until it is experimentally proved. In the Tertiary the different platan "species" may have been in contact! (Similar work is being done in Poland in order to clear up the origin of *Pinus uliginosa* (STASZKIEWICZ 1971 etc.).) But the author's remark that the recent platan species should be taxonomically redistributed by the principles of "numerical taxonomy" is considered to be a rash statement. Neither chemotaxonomy nor numerical taxonomy are able in themselves to create a new system. Acceptable results can only be attained by up-to-date geographical, ecological, genetical and morphological investigation methods — taking the peculiarities of the given plant into consideration (now this, now that method may be preferable).

As to the nomenclature of the recent platan species — considering that it is about a cultivated plant — *P. acerifolia* (Ait.) Willd. binom. can remain. Otherwise *P. orientalis* var. *acerifolia* Ait. may also be valid. Recently many taxonomic-botanists have endeavoured to rank lower taxons that they considered to be placed too high.

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ARE VEIN ANGLE DATA AS CONSERVATIVE MORPHOLOGICAL CHARACTERS SUITABLE FOR IDENTIFYING PLATAN VARIETIES?

The main value of the paper is the quantitative method the author elaborated by the utilization of mathematical statistics for the separation or identification of related fossil and recent species classified so far on the basis of subjective comparisons, thereby providing a possibility to clarify similar problems.

As a control we carried out angle measuring on 100 leaves of plane-trees *(Platanus acerifolia)* in the park of the Debrecen University. Four data were obtained from each leaf: the angles enclosed by the lowermost right and left lateral veins and by those above them with the midribs. In the above order they gave the following extreme values: 38—53; 33—54; 32—56; 31—54; with a standard deviation of 7. According to the results of measuring, the angles enclosed by the midrib and the mentioned four lateral veins are fairly constant, and so the vein angles as conservative morphological characters are suitable for the identification of the species. Therefore the author's conclusion — otherwise suggested by Andreánszky too — that *Platanus acerifolia* is no hybrid of *Platanus orientalis* and *occidentalis* but a progeny or survivor of *Platanus aceroides* Goeppert, a fossil species known from the Sarmatian layer, is considered to be correct.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 231—242 (1976)

LECTIONES

SALT BALANCE IN SALINE AND ALKALI SOILS*

All the properties characterizing salt affected soils develop due to the influence of salt solutions on the soil profile. Evidently, the study of salt movement and salt balance in these soils should be the first step towards a better knowledge of their nature and agronomical value. The salt regime determines not only the physical, chemical, physico-chemical and biological properties of salt affected soils, but their fertility and the possibilities of utilization and reclamation of these soils, as well.

The water soluble salts (mainly sodium salts) which cause soil salinity and/or alkalinity may occur either in the liquid or in the solid phases of the soil, or, most frequently, in both of them. Nevertheless, the salts affect the soil profile through the soil solution, due to the interaction of this solution with the other two phases (solid and gaseous phases) of the soils.

The quantity and the quality of soluble salts and their distribution in the soil profile are characteristic for the different types of salt affected soils. All systems of grouping and classification of these soils are based on various degrees and forms of their salinity and alkalinity. However, neither the total salt content nor its chemical composition, as well as the salt distribution in the soil profile are constant, on the contrary, they change periodically. One of the considerable seasonal changes was studied by JACKSON *et al.* (1956) and the data are shown in Table 1.

The table represents that, depending on the time of sampling, great differences can be found in the salt content of the soil horizons under saline conditions. In relation to soil alkalinity, similar regularities have been found by the same authors and many others (SZABOLCS—DARAB 1968).

Seasonal changes in the quality and quantity of salts in irrigated soils are shown in Fig. 1 (SZABOLCS 1969).

According to the figure not only the total amount of water-soluble salts changed in the studied soil profile, but their chemical composition and distribution in different horizons, as well.

It is evident from the above that in order to study the salt affected soils and to evaluate their properties for practical purposes, their salt content should be periodically measured, and the salt regime, as well as the salt balance of the soil should be characterized and determined. Evidently, the salt balance should be studied in close interrelation with the water balance of the soils.

The elements and types of salt balance in soils. The salt regime and salt balance of soils not only characterize the type and degree of salinization and alkalinization, but they give a basic knowledge for rational planning and utilization of irrigation and drainage systems, as

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* Introductory lecture delivered at the Symposium of the Subcommission of Salt Affected Soils of the International Society of Soil Science held in Cairo.

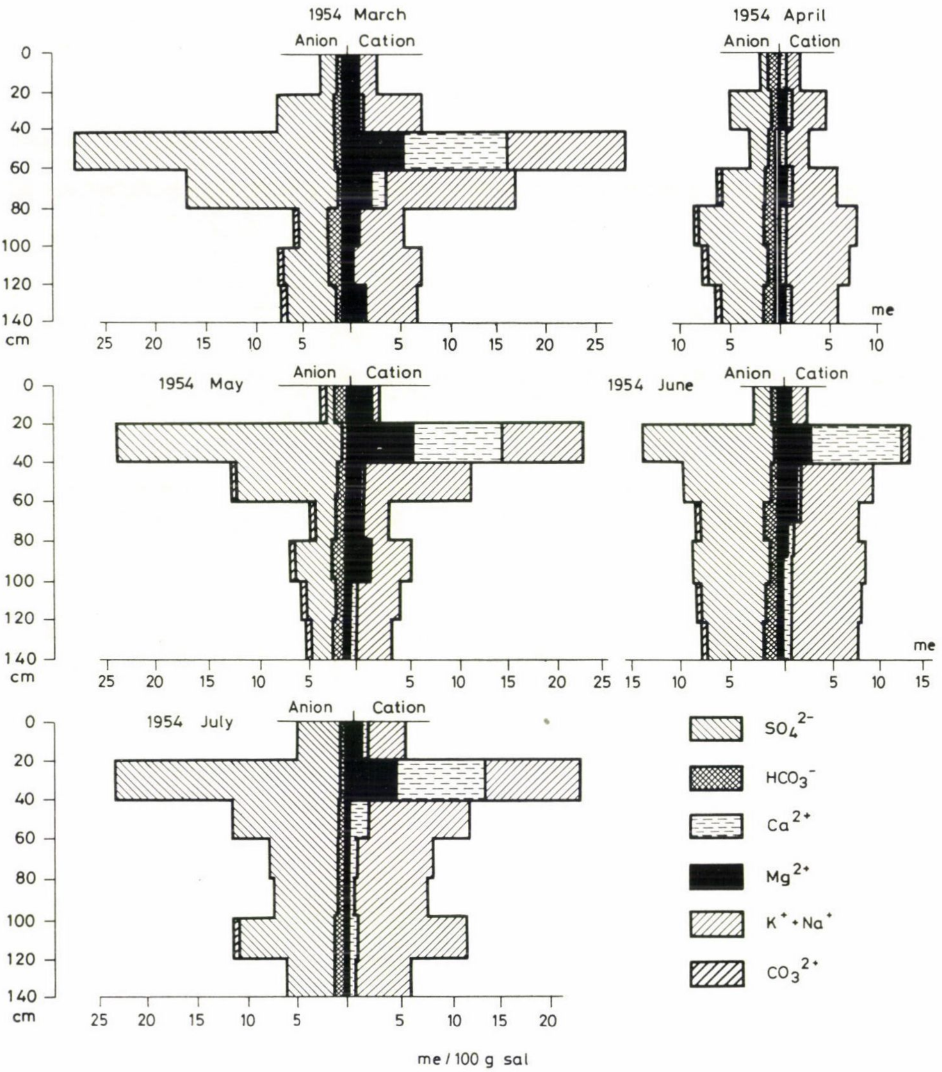

Fig. 1. Season dynamics of salt solutions (Szarvas, Hungary)

well. Parallel with the construction of new irrigation systems, the secondary salinization and alkalinization, as well as waterlogging of irrigated territories show a sharply growing tendency all over the world. Only a better knowledge of the dynamics of soluble salts, their actual and potential hazards may enable us to prevent serious damages caused by salinization in irrigated areas.

Kovda (1946) determined the following elements of salt balance:

1. Total amount of water soluble compounds in the soil
2. Influx of water soluble compounds into the soil
3. Outflow of water soluble compounds from the soil

Table 1

Seasonal variations in soil salinity in profiles at five sites, 1953—1954

(Salinity values estimated from the composite of six replicate samples (375 × specific conductivity of 1 : 5 soil suspension at 20 °C) by JACKSON *et al.* 1956)

Site	Sample	Texture*		Depth (cm)	Total soluble salts (% oven-dry soil)						
					July 1953	Sept. 1953	Nov. 1953	Jan. 1954	Mar. 1954	Apr. 1954	July 1954
1A	1	S	0—2.5	0—1	0.03	0.04	0.35	2.90	4.30	0.25	0.08
	2	S	2.5—15	1—6	0.02	0.04	0.31	0.51	0.53	0.27	0.04
	3	S	30—35	12—14	0.02	0.13	0.42	0.68	0.36	0.50	0.27
	4	S	45—50	18—20	0.20	0.21	0.63	0.66	0.50	0.61	0.65
	5	SCL	50—55	20—22	0.51	0.58	0.56	0.62	0.55	0.74	0.72
1B	1	S	0—2.5	0—1	0.03	0.04	0.03	0.28	0.33	0.07	0.03
	2	S	2.5—15	1—6	0.02	0.02	0.03	0.06	0.13	0.05	0.03
	3	S	30—35	12—14	0.02	0.03	0.10	0.11	0.09	0.05	0.02
	4	S	45—50	18—20	0.20	0.24	0.23	0.27	0.15	0.13	0.10
	5	SCL	80—85	32—34	0.56	0.57	0.55	0.57	0.48	0.41	0.47
1C	1	S	0—2.5	0—1	0.02	0.03	0.02	0.04	0.04	0.03	0.03
	2	S	2.5—15	1—6	0.04	0.02	0.02	0.02	0.03	0.02	0.02
	3	S	30—35	12—14	0.01	0.02	0.02	0.03	0.04	0.02	0.02
	4	S	60—65	24—26	0.06	0.04	0.12	0.12	0.10	0.04	0.02
	5	S	90—95	36—38	0.17	0.13	0.37	0.32	0.24	0.28	0.34
	6	SCL	110—115	44—46	0.36	0.39	0.50	0.40	0.38	0.32	0.47
2	1	L	0—2.5	0—1	0.06	0.15	0.88	1.60	1.10	0.16	0.13
	2	C	2.5—7.5	1—3	0.17	0.28	0.59	0.99	0.99	0.43	0.25
	3	C	17.5—22.5	7—9	1.00	0.58	0.94	1.50	1.60	2.20	1.50
	4	C	30—35	12—14	1.50	0.84	0.73	1.00	0.89	1.40	1.80
	5	C	45—50	18—20	1.00	0.84	0.69	0.81	0.66	0.72	1.40
	6	C	60—65	24—26	0.83	1.00	0.89	0.89	0.81	0.70	1.10
3	1	S	0—2.5	0—1	0.04	0.05	0.20	0.54	0.38	0.04	0.04
	2	S	2.5—15	1—6	0.03	0.05	0.16	0.13	0.10	0.05	0.03
	3	C	15—20	6—8	0.14	0.20	0.29	0.48	0.60	0.37	0.16
	4	C	45—50	18—20	0.66	0.59	0.72	0.71	0.75	0.95	0.79
	5	C	90—95	36—38	0.72	0.95	0.74	1.20	0.90	0.98	0.88

* S, sand. SCL, sandy clay loam. L, loam. C, clay

The quantity of salts in soils, as well as the increase and decrease in it can be expressed differently. However, more frequently, they should be calculated either in percentage or in p.p.m. or in tons per cubic metres of soil material. Depending on the purposes of determination they should be calculated either for several soil horizons, mainly for the top layers of the profile, or for the whole profile from the surface to the groundwater table. In all cases when the groundwater may be capillarily linked with the upper layers of soils, this calculation should be applied.

The general equation for salt balance by KOVDA (1946) is as follows:

$$\Delta S = S_2 + (S_{vw} - s_{vw}) + S_{iw} - S_v \tag{1}$$

where:

ΔS = change in total amount of salts
S_2 = total amount of salts at the beginning of determination
S_{vw} = influx of salts from groundwater
s_{vw} = outflow of salts into groundwater
S_{iw} = influx of salts from irrigation water
S_v = amount of salts taken up by plants

Depending on the value of ΔS, according to KOVDA (1946), DARAB (1962), SZABOLCS—DARAB (1968), SZABOLCS *et al.* (1969) three types of salt balance of soils may be distinguished:

1. Stable salt balance
2. Balance of salt accumulation
3. Balance of leaching

The salt balance may be established either for larger or for smaller territorial units, depending on the purpose of the study. For environmental purposes, general planning of irrigation and drainage systems, according to van der Berg, the whole river basin or watershed should be characterized and investigated, whereas for local irrigation and drainage execution the watershed or catchment area must be taken into consideration. Frequently, the salt and water balance of a small territorial unit or even one soil profile should be studied, especially for pedogenetic or fundamental research studies.

In the praxis of irrigation and drainage, one must always take into consideration that the surrounding areas permanently influence the salt balance of the given territory. On the other hand, the irrigation also alters the salt balance of the surrounding territories. Even successful irrigation often causes damages i.e. secondary salinization of soils, on the neighbouring fields.

The salt balance studies can be extended to the total salt content of soils if the aim is to study the overall salt regime, but in many cases the balances of certain ions or compounds should be studied separately. In alkali soils, in most cases, the latter method must be applied.

The climatic conditions definitely influence the type of salt balance. Under arid conditions, as a rule, the influx of salts; whereas under humid conditions the outflow of salts prevails. Beside climate, other elements of the environment, e.g. hydrology, relief, lithology, mineralogy also have a great influence on the salt balance of soils, furthermore, on the salt balance of certain areas. In salt affected areas the groundwater nearly always contains considerable amounts of soluble salts and the salinity of groundwaters represents one of the main sources of soil salinity. As long as the groundwater table is not linked with the upper layers of the soil profile, it is a potential possibility, but if they are linked, progressive salinization of soils takes place.

Irrigation has a very great influence on the environment, especially on the groundwaters and on their migration. As Kovda states, the groundwater affects the salt balance down to a depth of ten and even hundred metres, in case of intensive irrigation. In the groundwater balance he distinguishes the following classes:

1. Permanent, when its duration is measured in terms of geological periods, and it is connected with the geomorphological formation of the area.
2. Periodical (cyclic), usually lasting for approximately 11—25 years.
3. Annual, following the annual hydrological cycle of the area.
4. Inter-watering, lasting for the short period of time between the irrigations.

Table 2

Main types of groundwater regime in irrigated zones of the U.S.S.R. (Kovda)

Type	Sub-category	Probable trend of salt — accumulation processes
a. Stabilized, compensated (primary, secondary)	(1) Compensated by subterranean outflow	Desalinization. Little danger of secondary salinization
	(2) Compensated by subterranean outflow and transpiration	Desalinization $CACO_3$ and $CASO_4$ may accumulate in subsoil; secondary salinity may occasionally occur
	(3) Compensated by outflow, transpiration and evaporation	Slight salinization with accumulation of Na_2CO_3; Na_2SO_4; if methods of farming are poor, there may be strong salinization
	(4) Compensated by transpiration and evaporation	
	(5) Compensated by evaporation	Strong progressive salinization with accumulation of large quantities of NaCl, $MgCl_2$, $MgSO_4$, Na_2SO_4
b. Non-stabilized over-compensated	(1) Over-compensated by increase of feeding	When the groundwaters reach the critical level, an intensive, rapid process of salinization sets in, sometimes complicated by waterlogging
	(2) Over-compensated by decrease of outflow	
c. Non-stabilized under-compensated	(1) Under-compensated owing to decrease of feeding	With groundwaters around and above the critical level: decrease of salinity; with groundwaters below critical level: desalinization
	(2) Under-compensated owing to increase of outflow	Desalinization, increasing as the groundwater table drops

As regards the regime of groundwater, Kovda characterized the irrigated areas of the U.S.S.R. in Table 2.

As the table shows, the groundwater regime has similar elements as the salt regime of soils and their close interaction is also evident.

Irrigation water exerts a decisive influence on the salt balance of soils. The concentration and composition of the irrigation water determine whether its application results in either increasing or decreasing the salt content of the soil profile.

The hazard which is to be taken into consideration when evaluating the suitability of waters for irrigation has been determined somewhat differently in various countries. For instance, in the U.S.S.R. Kovda this evaluation is made as follows:

salt content g/l	evaluation
0.2—0.5	water of the best quality
1—2	water causing salinity hazard
3—7	water that could be used for irrigation only with leaching and perfect drainage

The standards set up by the U. S. Salinity Laboratory are shown in Table 3 (RICHARDS 1954).

Table 3

U.S. Salinity Laboratory's grouping of irrigation water

Classification of water	Electrical conductivity (micromhos per cm at 25°C) E. C.	Salt concentration in g/l (approximate)
C1 Low salinity water can be used for irrigation with most crops on most soils, with little likelihood that a salinity problem will develop. Some leaching is required, but this occurs under normal irrigation practices, except in soils of extremely low permeability	$0 < EC < 250$	< 0.2
C2 Medium-salinity water can be used if a moderate amount of leaching occurs. Plants with moderate salt tolerance can be grown in most instances without special practices for salinity control	$250 < EC < 750$	0.2—0,5
C3 High-salinity water cannot be used on soils with restricted drainage. Even with adequate drainage, special management for salinity control may be required and plants with good salt tolerance should be selected	$750 < EC < 2250$	0.5—1.5
C4 Very high salinity water is not suitable for irrigation under ordinary conditions but may be used occasionally under very special circumstances. The soils must be permeable, drainage must be adequate, irrigation water must be applied in excess to provide considerable leaching, and very salt tolerant crops should be selected	$2250 < EC < 5000$	1.5—3

The quality requirements of irrigation waters in Hungary are shown in Table 4.

Practically in all sytems for evaluating the quality of irrigation waters the main components to be determined are as follows:

Salinity (determined by electrical conductivity or by chemical analysis); Sodium percentage or SAR; Alkalinity. Ions of chloride, boron, magnesium, etc., should be determined according to the local circumstances and to different systems for the characterization of irrigation waters.

Beside groundwaters and irrigation waters there are numerous other factors influencing the salt balance of soils, such as soluble salts forming during the weathering processes in the soil, transport of salts by wind, seawater, etc. The chemical amendments, high doses of mineral fertilizers, as well as decomposition of plant tissues also have more or less influence on the salt balance of the given territory. As regards the outflow of salts from the soil profile, natural or artificial drainage plays a very important role and must be taken into consideration when the salt balance is to be measured or calculated.

Table 4

Quality norms of irrigation waters in Hungary

Suitability	Water type	Total salt mg/l	Na+ %	Phenolphthalein alkalinity, expressed in soda mg/l	Soda equivalent
A) Suitability in every case	*a)* hydrocarbonate	<500	35	10	
	b) hydrocarbonate-sulphate	<500	40	10	
	c) hydrocarbonate-chloride-sulphate	<500	45	10	
B) Suitable for certain soil types	*a)* hydrocarbonate	500—650	35	10	
	b) hydrocarbonate-sulphate	500—650	40	10	
	c) hydrocarbonate-chloride-sulphate	500—650	45	10	
	a) hydrocarbonate	650—800	30	10	
	b) hydrocarbonate-sulphate	650—800	40	10	
	c) hydrocarbonate-chloride-sulphate	650—800	40	10	
	a) hydrocarbonate	800—1000	35	10	
	b) hydrocarbonate-sulphate	800—1000	40	10	
	c) hydrocarbonate-chloride-sulphate	800—1000	45	10	
C) Suitable for certain salt affected soil types if irrigation is not connected with soil amelioration	*a)* hydrocarbonate	800	35—65	10—50	
	b) hydrocarbonate-sulphate	800	40—75	10—50	
	c) hydrocarbonate-chloride-sulphate	800	40—75	10—50	
	a) hydrocarbonate	1000	35	50	
	b) hydrocarbonate-sulphate	1000	40	50	
	c) hydrocarbonate-chloride-sulphate	1000	45	50	
D) Suitable in every case after chemical amelioration	*a)* hydrocarbonate	300	40	50—100	2.0—3
	b) hydrocarbonate-sulphate	300	45	50—100	2.0—3
	c) hydrocarbonate-chloride-sulphate	300	50	50—100	2.0—3
E) Suitable for certain soil types after chemical amelioration	*a)* hydrocarbonate	300—500	40	100—200	3—6
	b) hydrocarbonate-sulphate	300—500	45	100—200	3—6
	c) hydrocarbonate-chloride-sulphate	300—500	50	100—200	3—6
F) Suitable in every case if improved by dilution	*a)* hydrocarbonate	500—1000	60	10—30	
	b) hydrocarbonate-sulphate	500—1000	60	10—30	
	c) hydrocarbonate-chloride-sulphate	500—1000	60	10—30	
G) Suitable for certain soil types if improved by dilution	*a)* hydrocarbonate	1000—2000	70	10—50	
	b) hydrocarbonate-sulphate	1000—2000	70	10—50	
	c) hydrocarbonate-chloride-sulphate	1000—2000	70	10—50	

The application of salt balance studies for prediction and control of secondary salinization and alkalinization of irrigated areas. The limit values of the chemical composition of irrigation water, characterizing its suitability, can be determined regionally, according to the given environmental conditions (drainage, climate, properties of soils, salt tolerance of cultivated plants, etc.). That is the reason why very different norms of water quality exist in different areas.

As regards the groundwater table, after POLINOV (1956) and KOVDA (1946) the term of "critical depth of the groundwater table" has been introduced. If the groundwater table is below the "critical depth", the total salt content of the soil profile above the groundwater table is diminishing, because the salts move permanently into the groundwater from the soil profile, due to downward solution movement. If the groundwater is above the "critical depth" a permanent increase in the salt content of the soil profile can be observed, due to the upward movement of salt solutions. Depending on the intensity of the above mentioned processes, sooner or later remarkable changes take place in the salinity and/or alkalinity of soils.

As regards the interpretation of the "critical deph of the groundwater table", two main approaches should be distinguished:

1. The area has a perfect drainage system. In this case permanent or periodical leaching can prevent the accumulation of harmful salts in the root zone of plants i.e. in the upper layers of the soil profile. Under such conditions the groundwater table should be controlled and kept at a suitable depth, to ensure sufficient protection for the crop roots and topsoil horizons against high salinity and/or alkalinity.

This is the praxis in most of the arid countries, where any irrigation affects salinization progressively and a systematic leaching of irrigated fields through a drainage system is unavoidable.

2. In several areas with poor natural drainage and semi arid or semi humid climatic conditions the subsoils and/or groundwaters contain considerable amounts of harmful salts, but as long as the groundwater is below the critical level, they cannot rise and damage the soil profile. In case of intensive irrigation in such areas the groundwater table rises, but through a firm control of the density of irrigation and irrigation water filtration, the salinization and/or alkalinization of soils can be prevented. Irrigation should be applied in such cases until the groundwater table rises to the critical depth. If it is rising above it, the construction of a drainage system or the reduction of irrigation is necessary.

If the water table is at the critical depth the salt balance in the soil profile above the groundwater is in equilibrium. Evidently, by salt balance studies and equations the numerical values of recent and predicted salinity of soils, as well as the critical depth of groundwater can be determined or approximated.

All the equations used recently for practical purposes in the field of salt balance studies of soils are empirical. In spite of many valuable results in the field of soil physics and chemistry, (for instance: TANJI *et al.* 1967a, b, TSALMA–PHILIP 1971), we still lack the exact parameters for calculating the salt dynamics in the soil profile for the praxis of irrigation and drainage.

The elaboration and application of empirical equations for salt balance determinations require regular field and laboratory analyses of water, soils, etc., in the given place. Under irrigated conditions the salt regime of soils depends on the method and the technical level of irrigation, as well. These should be also taken into account when using the above mentioned empirical approaches.

We elaborated the following equation for the practical determination of the salt balance of soils under the conditions of irrigation:

$$b = a + \left(d + \frac{CV_n}{Mt_{f_s}} \cdot 10^{-5}\right) \tag{2}$$

where:

b = soluble salt content of the soil at the end of observation, mg/100 g soil
a = soluble salt content of the soil at the beginning of observation, mg/100 g soil
c = salt concentration of the irrigation water, g/l
v = quantity of the irrigation water applied during the observation period, m^3/ha
M = thickness of the soil layer for which the salt balance was established, m
t_{fs} = bulk density of the soil
d = salt regime coefficient of the soil, g/100 g soil

The salt regime coefficient gives the change that occurred in the salt content of the soil during the observation period. Thus it gives the difference between the amount of salts leached from the soil and that of salts got into the soil from sources other than the irrigation water.

It is rather difficult to calculate the value of "d", so we had better determine the change in the salt content of the soil and calculate the salt regime coefficient on the basis of the following correlation:

$$d = b - \left(a + \frac{cv}{Mt_{fs}} \cdot 10^{-5}\right). \tag{3}$$

According to the examinations, the salt regime coefficient of a given soil type is constant in a given area if the drainage conditions do not change essentially, so it is suitable to characterize the salt balance of soils and the factors influencing the former. Having completed the calculations for several irrigated soils, we could distinguish the following characteristic cases:

a) The salt regime coefficient is of a negative value, the salt content of the soil decreased during the observation period. Thus consistent leaching took place in the soil.

b) The salt regime coefficient is of a negative value, but the salt content of the soil increased during the the observation period. This means that although leaching took place to a certain extent, more salt accumulated in the soil from the irrigation water than that could be leached out. Thus the salt content of the soil increased and the irrigation water was the source of salts. The following measures may be taken to change the salt regime of the soil: the same amount of irrigation water may be applied but its salt concentration must be reduced; the amount of irrigation water may be reduced; the same amount of irrigation water of the original salt concentration may be applied but then drainage of the area, the possibilities of leaching must be improved.

c) The salt regime coefficient is of a positive value and the soluble salt content of the soil increased during the observation period. This indicates that more salt accumulated in the soil — not only from the irrigation water but also from the groundwater and perhaps from other sources of salts — than that could be leached out. In this case the source of the salts is the saline subsoil and saline groundwater. The salt balance becomes negative only if the drainage conditions of the area and the water regime properties of the soil are improved.

d) The salt regime coefficient of the soil may be zero indicating that the amount of salts accumulated in the soil from various sources is equal with the amount leached out. In this case the salt balance of the soil is stable.

In order to forecast the change in the soil's salt content due to irrigation, equation (3) can be used.

For the calculation of the maximum permissible salt concentration with which the soil's salt content would not be changed by irrigation (a = b) equation (3) is used in the following form:

$$c = \frac{dMt_{fs}}{v \cdot 10^{-5}}. \tag{4}$$

The change in the soil's salt content to be expected if a certain amount of irrigation water of a given salt concentration is applied, can be calculated by using the same equation in the following form:

$$b = a + \left[d + \frac{cv_n}{Mt_{fs}} \cdot 10^{-5}\right]. \tag{5}$$

From the data presented here, it can be seen that the total salt content of the irrigation water is of general descriptive value only, and the limit values established in one classification system are not applicable without restriction everywhere and under every condition.

In our calculation method, given below, for the determination of the critical depth of the water table we assume that:

a) The groundwater rises to the capillary zone of the soil and the soil solution migrates within the soil profile without changes in their salt concentration and composition.

b) The soil solution in the whole soil profile is saturated with Ca and Mg salts and practically only sodium salts migrate within the soil profile. The capillary flow (amount of water rising from the groundwater into the unsaturated soil layers) was taken as a simple function of the depth to the water table. It was a rather rough estimation because, for the exact determination of the capillary flow, at least two more factors must be taken into account: capillary conductivity as a function of suction, and the suction profile. Substitution of measured values for these factors instead of the estimated averages would improve the calculation considerably. Furthermore, other factors affecting the capillary flow (precipitation, evaporation, soil texture, structure, porosity, water holding capacity, permeability, available moisture content, etc.) may be taken into consideration because of their determining or modifying effect on capillary conductivity or on the suction profile. The effect of human activity (irrigation, melioration, agrotechnics, etc.) on capillary rise and, consequently, on the water and salt regimes of soils may also be evaluated.

In our present calculation we took the following factors as variables:

a) Amount of irrigation water

b) salt content of irrigation water

c) "salt regime coefficient", or the annual salt balance of unirrigated soils, i.e. the quantity of salts leached out or accumulated per year

d) thickness of the capillary zone

e) categories of soil water properties (volume weight, water holding capacity, available moisture content and permeability)

f) average total salt content of the soil between the surface and the water table

g) salt content of the groundwater

We used calculation method "a" if the Na percentage of the irrigation water was below 75, or the pH in the B_1 horizon was below 9, and method "b" if the Na percentage of the irrigation water was above 75, or the pH in the B_1 horizon was above 9.

The consecutive steps of the calculations in the "a" method are given below:

$$x = \frac{c_1 \cdot w_1}{D_1 \cdot v} \cdot 10^{-5} \tag{6}$$

x = quantity of salts added by the irrigation water, per cent of soil

c_1 = concentration of the irrigation water, g/litre

w_1 = amount of irrigation water used, m^3/hectare

D_1 = thickness of the soil layer between the surface and the capillary fringe (upper boundary of capillary zone), m

v = volume weight of the soil, g/cm^3

$$y = \frac{c_2 \cdot w_2}{D_2 \cdot v} + a \tag{7}$$

y = quantity of salts in the capillary zone, per cent of soil
c^2 = concentration of the groundwater, g/litre
w_2 = quantity of availabe moisture in the capillary zone, m^3/hectare
D_2 = thickness of the capillary zone, m
a = average original salt content of the soil before irrigation, per cent of soil

$$c_3 = \frac{y}{W_k} \cdot 10^{-3} \tag{8}$$

c_3 = concentration of the soil solution in the capillary zone, g/litre
W_k = field capacity, weight per cent

$$z + \frac{c_3 \cdot w_3}{D_1 \cdot v} \tag{9}$$

z = quantity of salts transported by capillary flow from the groundwater and the capillarily saturated soil layers to the unsaturated zone of the soil, per cent of soil
w_3 = capillary flow (amount of water rising from the groundwater into the unsaturated soil zone, m^3/hectare)

$$b_{10} = (a + x + z + d) \cdot 10 \tag{10}$$

b_{10} = average salt content of the soil after 10 years' irrigation (assuming that the above mentioned factors are constants during this period), per cent of soil

$$b_{10} - a = (x + z + d) \cdot 10 \tag{11}$$

d = "salt regime coefficient" i.e. the quantity of salts leached out or accumulated under natural conditions per year, per cent of soil
$b_{10} - a$ = change in the average salt content of soil (salt balance) due to ten years' irrigation, per cent of soil

The calculation procedure of the "b" method is the same except that only the equivalent quantity of sodium (alkali) salts in the irrigation water, groundwater, soil solution and the "salt regime coefficient" rather than the total soluble salt content must be calculated.

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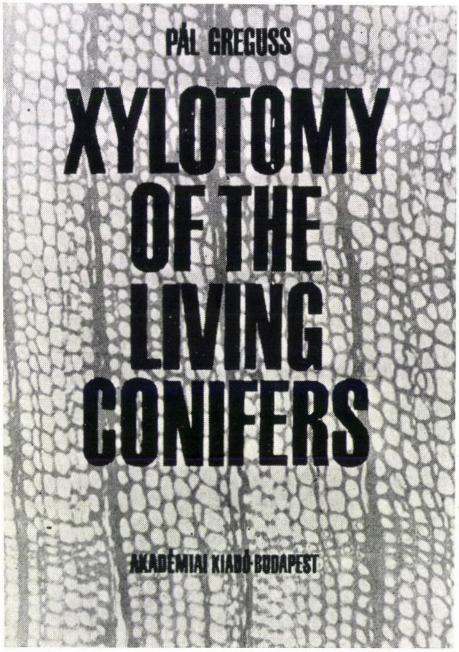
Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1—2), pp. 243—247 (1976)

RECENSIONES

P. Greguss: *Xylotomy of the living conifers.* Akadémiai Kiadó, Budapest, 1972. pp. 172., 808 microphotos on 175 plates, and 145 plates with drawings.



This is the tenth important work on xylotomy the author, well-known in professional circles all over the world, has published since 1947 in the Hungarian, German and Russian languages. The book is the continuation and completion of the author's work "Identification of living Gymnosperms on the basis of xylotomy" (Akadémiai Kiadó, Budapest) published in 1955 in English and German.

This earlier monograph which bore witness to an enormous work, if only by its size, was highly appreciated all over the world both for its content and get-up. The reputed foreign experts of xylotomy declared in various journals that it was a long-needed comprehensive work unparallelled so far in the literature of the profession. Here in Hungary it was qualified as the "finest book of the year" and its author was awarded the silver medal of the Kossuth Prize. On the international book exhibition in Leipzig it was also considered an outstanding work and as such met with the highest recognition.

The value of the author's activity is greatly increased by the fact that xylotomy, i.e. the microscopic study of the tissue structure of trees and the identification of the species on the basis of microscopic study, promotes research in other theoretical and practical sciences to a very great extent, often even decisively. The identification of several million years old fossile tree remnants originating from earlier geohistorical eras often supplies indispensable data for paleobotany, phylogenetics, phytogeography, geology and meteorology too. The work of historians and archeologists is also greatly supported by xylotomy. Namely, during the excavations many wooden instruments, articles for personal use and other wooden remnants (tools, vessels, coffins, etc.) are found whose identification contributes to the knowledge of the historical and prehistorical times. From the charcoal remnants originating from the fireplace of the prehistoric man important conclusions can be drawn on the arborescent vegetation of the glacial and postglacial eras, in the first place. Beyond what have been mentioned, practical life also calls for the work of xylotomists. In forest management, trade and some industries, like paper and furniture industry and other branches of wood industry, the histological study of trees is indispensable. Moreover, it is often required in the course of legal procedures.

In his earlier work published in 1955 the author wrote up 350 of the about 520—530 Gymnospermous species at present living on the earth, that is 66 per cent of the total number of living species. He gave a detailed xylotomic description, four fine and highly illustrative microphotos and a plate with drawings of each species examined.

In his last work published in 1972 the author discusses the wood anatomy of further 160 pine species missing from the earlier book, which represent 34 per cent of the *Gymnospermae* living at present. So these two monographs together contain the full xylotomic treatment of the living Gymnospermous plants, and as such are unmatched in the professional literature.

The author is in connection with many botanic and forestry institutes, arboretums, museums of the five continents and with the experts working in them; he received the material required for his work from them. The material of the 160 pine species required for the recently published volume was mostly obtained from the following countries. England, German Democratic Republic, German Federal Republic, Belgium, Portugal, Roumania, Holland, Soviet Union, Poland, France, Spain, China, Japan, Indonesia, Taiwan, New-Guinea, Sarawak, Australia, United States of America, Cuba, Malaysia, Philippines, Mexico, Argentina, New-Caledonia, Chile, New-Zealand.

The volume published in 1972 which — as we have mentioned — contains the xylotomy of 160 living pine species missing from the earlier work is divided as follows: After the preface and the acknowledgements a short introduction surveys the types of woody plants from the point of view of the structure of the wood. Further on the book contains a shorter general and a large specific part.

In the general part the major xylotomic properties of the *Cycadinae* class are first outlined mainly to compare it with the *Coniferae* class. Subsequently it gives a short xylotomic survey of the families *(Araucariaceae, Podocarpaceae, Taxales, Cupressaceae, Taxodiaceae, Pinaceae)* belonging to the *Coniferae* class.

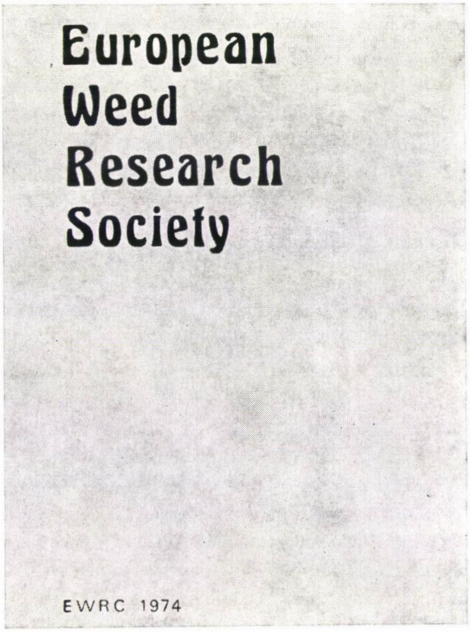
The specific part which contains detailed descriptions of 160 species missing from the author's previous work begins similarly with the *Cycadinae* class and within it gives descriptions of species belonging to the families *Cycadaceae, Stangeriaceae, Zamiaceae.* The largest part of the work is occupied by the discussion of the *Coniferae* class. Species belonging to the following families and genera are described here: of the *Araucariaceae* family the species of the *Agathis* and *Araucaria* genera; of the *Podecarpaceae* family the species of the *Dacrydium, Microstrobus, Phyllocladus* and *Podocarous* genera; of the *Taxaceae* family *Taxus floridana;* of the *Cupressaceae* family species belonging to the *Callitris, Cupressus, Heyderia, Juniperus* and *Libocedrus* genera; of the *Taxodiaceae* family species belonging to the *Athrotaxis, Cryptomeria* and *Cunninghamia* genera; and finally, of the *Pinaceae* family species belonging to the *Abies, Larix, Picea, Pinus, Cathaya, Pseudotsuga,* and *Tsuga* genera. The description of the individual species begins with the precise indication of the site of origin, then the anatomic characteristics of cross section, radial longitudinal section and tangential longitudinal section are described in detail, with quantitative data relative to the dimensions of the xylem elements. We have to emphasize that all these descriptions are based on the author's own investigations and observations. The book is completed by a bibliography and an index.

The illustrations of the work consisting of microphotos and microscope drawings have to be treated separately. The 10 plates belonging to the general part illustrate — partly in stereoscopic pictures — the features characteristic of the anatomic structure of the trunk, and within it of the xylem of the *Gymnospermae.* The 155 photographic plates belonging to the specific part present the xylotomic properties of the species disscussed by one cross-section, one radial longitudinal section and two tangential longitudinal section microphotos made of each species. Beside the photographic plates 145 microscope drawings illustrate the most typical xylotomic features of the species. Both the microphotos and the microscope drawings are unobjectionable from a professional point of view; they excellently show the xylotomic characters of the discussed species, and in addition their printing technical quality is also outstanding.

To sum up, we can say that Prof. Greguss' book on the "Xylotomy of the living Conifers" is an excellent work regarding both its content and get-up. And together with the earlier published "Identification of living Gymnosperms on the basis of xylotomy" it is a unique work supplying a great want in the world literature on xylotomy.

L. Fridvalszky

European Weed Research Society. EWRC 1974 Oxford



European
Weed
Research
Society

EWRC 1974

The European Weed Research Council (EWRC) announced last November that it is to be replaced by a new international society whose membership will be open to all individuals interested in weeds and weed control. Since 1960 the Council, which is based on a membership of one weed specialist from each of some 28 countries, has done much to enhance the status of weed science in Europe and elsewhere. As the first international regional organization concerned with this subject to be formed it has become well known throughout the world for its sponsorship of the journal Weed Research and for the many successful international symposia it has organized in various European countries. Other activities of EWRC have included: the formation and communication between workers in different countries, sponsorhip of training and refresher courses for weed specialists, promotion of weed science teaching and research at universities, production of a directory of weed research workers in Europe and Summer Meetings combined with field excursions and local seminars in member countries. These latter have been particularly valuable in providing opportunities to develop scientific, cultural and personal links between the EWRC members from some 28 countries and weed scientists throughout Europe.

Whilst EWRC has accomplished much, its role has been severely limited by its membership restricted to one specialist from each country, also by lack of funds. Also industry has been largely excluded. It is hoped that by removing the first and last of these limitations the new Society will attract many new members from official research stations, from universities and from industry and that these in turn will greatly enhance the range of activities and in due course the financial status of the Society. The Society will be controlled by a Council based on one national member from each country and elected representatives of the Society's membership, an equal number of whom will come from industry and other organizations. The President and Vice-President are also to be elected by the membership. The offices of President and Vice-President will alternately be filled by a non-industrial member and a member from industry. At any one time either the President or the Vice-President will be a member from industry.

The European Weed Research Society was formed on 1st January 1975 under a Steering Committee set up by EWRC.

The European Weed Research Society (EWRS) is a new international society open to all who are interested in weeds, weed control and related topics. Its object is to encourage and promote weed research and technology in Europe for the advancement of agriculture and the benefit of the community as a whole.

The Society, as described in the Constitution, will: promote and assist communication between individuals and organizations; advise, collaborate and maintain liaison with relevant national and international organizations; identify problems of weeds, weed research and technology and stimulate research and other endeavours to overcome them; organize meetings, symposia and conferences; collect and disseminate information; encourage and assist education and training.

It is the intention that the Society will adopt the achievements, assets and activities of EWRC and with its far greater manpower resources develop and add to them.

Detailed information about EWRC and EWRS, together with membership application forms, is available in a brochure written in English, French or German, obtainable free of charge from the following:

ENGLISH: J. D. Fryer · ARC Weed Research Organization · Begbroke Hill, Yarnton Oxford OX5 1PF · United Kingdom

FRENCH: R. Faivre-Dupaigre · 8 Av du President-Wilson · 75116 · Paris · France

GERMAN: J. H. Gortz · IBS Weed Control Department · PO Box 14 · Wageningen · Netherlands

Participation in EWRS is not restricted to those living in Europe. It is hoped that many existing national and regional organizations concerned with weed science will wish to become affiliated to EWRS.

B. Győrffy

D. Hámori: *Hereditary constitutional defects and diseases of domestic animals.* Akadémiai Kiadó, Budapest, 1974. 583.

The development of livestock breeding calls for a wide application of the most up-to-date selection methods and breeding techniques. That is why progeny tests, the planning and organization of breeding providing a great selection-pressure, as well as in certain domestic animal species a reasonably applied inbreeding and line breeding play an ever increasing role. These methods of increasing the volume and economic efficiency of production are necessarily accompanied by the more frequent occurrence of the genes that form the genetic basis of the standard qualities that are expected to improve. At the same time, however, beside the genes predetermining the desired properties a more frequent manifestation of harmful factors representing a genetic abnormality must be reckoned with too. In this respect a particularly great danger may arise from the overfavorization of a sire by artificial insemination, if it carries the recessive gene or genes of some genetic abnormality in a heterozygous state. The fact that in consequence of the rapid development of the technique of sperm preservation the hereditary properties of any highly qualified male can be propagated in time and space almost boundlessly further increases the importance of disclosing and studying the genetic abnormalities and exercising a systematic control over them. In the future we have to prepare ourselves to increased economic losses caused by the manifestation of genetic abnormalities. The prevention of these losses is the joint task of animal breeders and veterinary surgeons specially trained in genetics. The organs and institutions of breeding management have a very great responsibility in this field, with special regard to the international integration of breeding work (exchange of breeding animals and sperm, establishment of sperm banks etc.), and exploration and utilization of gene reserves.

From the above it is clear that the Author — who was entitled by his wide knowledge of the special literature, several decades of practical experiences and experimental investigations to write this work — undertook an extremely important, timely and involved task when preparing the manuscript of this comprehensive, pioneering work of the Hungarian special literature.

The book runs to 51.1 printed sheets (A/5) with a large number (221) of illustrations, and is divided into the following main chapters:

- I. General part (1.1. Introduction, 1.2. Importance of genetic pathology in livestock breeding, 1.3. Transmittance of properties reducing production, 1.4. Necessity of sorting out the pathological properties, and the breeding hygienic directives of selection, 1.5. Frequency of inborn pathological properties, 1.6. Lethal losses, 1.7. Hereditary predisposition to diseases, 1.8. Prevention, identification and elimination of hereditary constitutional abnormalities in livestock, 1.9. Role of artificial insemination in preventing the spread of hereditary pathological properties).
- II. Cytogenetical and clinical relations of constitutional abnormalities.
- III. Fertility and prolification.
- IV. Hereditary disturbances of reproduction in female domestic animals.
- V. Hereditary abnormalities of metabolism.
- VI. Genetic pathology of organs and organ systems.
- VII. Gastrointestinal tract.
- VIII. Respiratory diseases.
- IX. Circulatory system.
- X. Renal diseases.
- XI. Breeding hygiene of the mammary system.
- XII. Organs of locomotion.

- XIII. Susceptibility and resistance to diseases.
- XIV. Appendix (Population genetic calculations, registration of abnormalities, veterinary management- and registration measures, list of lethal abnormalities. Name and subject index).

The subchapters are generally completed by detailed references.

For the very reason that it must be regarded as a pioneer undertaking in the Hungarian literature on livestock breeding and veterinary science, Hámori's work certainly excites great interest and gives rise to discussions in the special circles. The expected discussions can by all means be useful and may promote an organized protection against genetic abnormalities.

The readers of the work are: György Kiszely, Andor Kardeván and János Dohy.

For the attractive make-up of the book the Publishing House and Printing House of the Hungarian Academy of Sciences deserve praise.

J. Dohy

Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (1–2), pp. 249–252 (1976)

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INDEX

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A kiadásért felel az Akadémiai Kiadó igazgatója. Műszaki szerkesztő: Botyánszky Pál
A kézirat nyomdába érkezett: 1976. I. 6. — Terjedelem: 24,5 (A/5) ív 86 ábra

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Index: 26.001

ACTA AGRONOMICA ACADEMIAE SCIENTIARUM HUNGARICAE

TOMUS XXV FASCICULI 3–4

AKADÉMIAI KIADÓ, BUDAPEST
1976

ACTA AGRON. HUNG.

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Megrendelhető a belföld számára az Akadémiai Kiadónál (1363 Budapest Pf 24. Bankszámla 215-11488), a külföld számára pedig a ,,Kultúra" Könyv- és Hírlap Külkereskedelmi Vállalatnál (1389 Budapest, 62, P. O. B. 149 Bankszámla: 218-10-990) vagy annak külföldi képviseleteinél és bizományosainál.

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INDEX

Fasc. 1—2

VARIA

FORUM

CONTRIBUTIONS

LECTIONES

RECENSIONES

AUCTORES

Fasc. 3–4

VARIA

LECTIONES

FORUM

CONTRIBUTIONS

RECENSIONES

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РЕЗЮМЕ

ВЛИЯНИЕ СИНТЕТИЧЕСКИХ АМИНОКИСЛОТ И ДОБАВЛЕНИЕ КАРБАМИДА НА ОТКОРМ ЯГНЯТ

М. СЕЛЕНИ ГАЛАНТАИ, Й. ЕЧАИ, Б. ЮХАС

В опыте изучали влияние добавления dl — метионина и 1 — лизина к кормовой смеси, а также возможность заменения препаратом, экстрагированным из соевой крупы, с прибавлением карбамида (НИИ Месличных Культур и Моющих Средств) на откорм рано отделённых ягнят. Использованная кормовая смесь содержала переваримого сырого белка 12%, крахмала 72—75 кг/ц. Опыты были проведены с мериносовыми мясо-гибридными ягнятами в 6-и группах, в каждой по 12 баранов и ярок. Самый хороший результат получен в группе, корм которой не содержал сои, последняя была заменена супер-концентратом с добавлением карбамида, 0,4% dl — метионина и 0,3% 1-лизина. В этой группе привес был на 13% больше контроля, а использование корма на 6%. Если к кормовой смеси, содержащей соевую крупу, добавляли 0,3% лизина, привес повышался на 8% и на 9% понижалось количество кормовой смеси, затраченной на привес в один кг. В начале и в конце эксперимента брали кровь у животных с целью определения в плазме содержания общего белка, аминокислот, MN, лизина и метионина. Изучение содержания M плазмы, учитывая привес животных, даёт полезные данные, — относительно встроения азота, — по снобжению животных белком. Так изчуение содержания MN в кровяной плазме явилось хорошим индикатором изменения обмена азота в процессе эксперимента. Концентрация лизина и метионина в плазме не следовала за добавленным к корму количеством синтетических аминокислот, что до сих пор наблюдалось в опыте со свиньями.

КОНЦЕНТРАЦИЯ МАКРО- И МИКРОЭЛЕМЕНТОВ СЕМЯН ОВОЩНЫХ РАСТЕНИЙ, ПРИНАДЛЕЖАЩИХ К СЕМЕЙСТВУ CRUCIFERAE

ДЬ. ТЭЛДЕШИ, Т. МАЙОР

Анализируя 169 семенных образцов по 11 элементам овощных растений, принадлежащих к семейству крестоцветных, можно было установить, что их самым характерным свойством является высокое соотношение P/Cu, по которому они могут быть отличены от семян всех овощных, возделываемых в значительном количестве в Средней Европе Концентрация минерального вещества семян сортов с более длительным вегетационным периодом была ниже по тенденции, чем у сортов с коротким вегетационным периодом.

ИЗУЧЕНИЕ ЭФИРНОМАСЛИЧНОЙ ВЫДЕЛИТЕЛЬНОЙ СИСТЕМЫ ЛИСТЬЕВ У ЭСТРАГОНА (ARTEMISIA DRACUNCULUS L.)

Ж. ЛАШШАНИ, ДЬ. ШТИБЕР

Авторы изучали эфирномасличную выделительную систему настоящего листа у эстрагона (*Artemisia dracunculus* L.) в течение онтогенеза гистологически и гистохимически, но были проведены и опыты по тонкослойной хроматографии. Установили, что желёзки не содержат метилкавикол; количество этого соединения зависит от возраста листьев.

ВЛИЯНИЕ РАЗНЫХ ДОЗ КАРБАМИДА НА ОБМЕН ВЕЩЕСТВ ЖВАЧНЫХ

Е. СЮЧ, А. РЕГИУС-МЭЧЕНИ

Авторы изучали влияние трёх разных доз карбамида, исследованных при кормлении, на видимое использование питательных веществ различных кормовых доз и на азотообмен опытных животных, которыми служили бараны и бычки венгерской пестрой породы по 36 шт. в каждом стаде. В результате опытов разное количество карбамида, дававшееся баранам ежедневно в дозе 20, 30 и 40 г на 100 кг живого веса, а бычкам 25, 37,5, и 50 г карбамида на 100 кг живого веса, не оказали заметного влияния на использование питательных веществ и на азотообмен подопытных животных. На основании опытов — после соотвествующего приучения — кажется целесообразным стремление повысить пропорцию сырого белка карбамида в пределах содержания сырого белка кормовых доз. Опыт показал, что пропорция карбамида может быть повышена и в кормовых дозах развивающихся бычков без понижения использования белков и других питательных веществ а, также без уменьшения задержки азота.

ГИСТОХИМИЧЕСКОЕ ИЗУЧЕНИЕ ЭНДОГЕННОГО ПАРООБРАЗОВАНИЯ У ПЛОДОВ ЯБЛОНИ СОРТА ЙОНАТАН

Т. БУБАН, Й. МАЦ

Изучались гистологические и гистохимические особенности парообразования в плоде яблони сорта Йонатан. Соотвествующие ткани плода тестировались с помощью бактерий и грибов. На основании разнообразных внешних морфологических признаков, отрицательного результата микробиологического теста, а также гистологических особенностей не могли определить причину ненормальности.

ИЗУЧЕНИЕ СВОБОДНЫХ АМИНОКИСЛОТ ВИНОГРАДНЫХ ЯГОД С ПОМОЩЬЮ ФИКСИОН 50×8, СОДЕРЖАЮЩЕГО СМОЛЫ ДЛЯ СИЛЬНОГО ОБМЕНА КАТИОНОВ

О. ЮХАС, Д. ПОЛЯК

У трёх сортов винограда *(Vitis vinifera* **L., cultivar Rizlingszilváni Synn. Müller Thurgau, Olasz rizling Syn. Wälschriesling, Piros tramini Syn. Gewürztraminer)** определили содержание свободных аминокислот в ягодах хроматографическим расслоением с помощью **Фиксион** 50×8, содержащего смолы для сильного обмена катионов. Удалось освоить аналитический метод, с помощью которого можно в одной димензии разделить 16—17 аминокислот, что позволяет произвести одновременно быстрое сравнение нескольких проб при серийных анализах. Используя данный метод, провели сравнительное исследование, касающееся изменения состава свободных аминокислот, в зависимости от созревания контрольных и обработанных азотным удобрением образцов, которые выращивались в поле и в вегетационных сосудах. В виноградном соке в процессе созревания и под влиянием азотного удобрения, больше всего накапливалось пролина и аргинина.

ПРОДУКТИВНОСТЬ НЕКОТОРЫХ ГЕНОТИПОВ ЛЮЦЕРНЫ В УЗКО- И ШИРОКОРЯДНЫХ ПОСАДКАХ

1. Наследуемость фуражного урожая и родственных признаков и взаимоотношения между ними

А. М. РАММАН, З. БЭЙТЭШ

Цель этого исследования — определение наследуемости важных признаков люцерны с разным типом развития в узко- и широкорядных посадках. Использованные 4 клона представляли прямостоячий, полустоячий, полустелющийся и стелющийся типы развития. Семена, полученные от ветроопыления 4 клонов, были посеяны, и растения, отселектированные на основании самого высокого урожая сухого вещества, были размножены вегетативным путём и пересажены на опытное поле, применив два различных междурядий. Оценка наследуемости разных признаков в узком смысле слова по сумме трёх кошений была получена путём дисперсионного анализа. Коэффициенты фенотипической и генетической корреляции были вычислены из фенотипической и генотипической ковариансы. Значения наследуемости высоты растений были 112, 107 и 97% в широкорядных посадках, 37, 61 и 44% в узкорядных посадках у клона С-37, С-636 и С-1474. Наследуемость по числу стеблей на растение у клонов С-244, С-636 и С-1474 была выше в широкорядной посадке. Наследуемость по урожаю сухого вещества и урожаю сухих стеблей была ниже в широкорядной посадке у клонов С-37 и С-244, обратные оценки были выявлены у двух других клонов. Оценка наследуемости по весу сухих листьев была приблизительно одинакова в обоих системах посадки за исключением стелющегося типа (С-1474), который был выше в широкорядной посадке. Большинство фенотипических корреляций между свойствами были положительными и достоверными в двух системах посадки. Более сильные ассоциации были получены между высотой растения и зеленым урожаем, урожаем сухого вещества и урожаем сухих стеблей, урожаем сухого вещества и урожаем сухих листьев в узкорядных посадках.

ВЫЖИВАНИЕ, РАЗВИТИЕ КУКУРУЗНОГО МОТЫЛКА (EUROPEAN CORN BORER) И ПОВРЕЖДЕНИЕ ИМ РАСТЕНИЙ «OPAQUE–2» И НОРМАЛЬНОЙ КУКУРУЗЫ

М. Б. ВИНДЕЛС, Х. Ц. ЧИАГ

Сравнивались выживание, развитие кукурузного мотылка **Ostrinia nubilalis (Hbn.)** и повреждение им растений у **«opaque-2»** и их нормальных аналогов, используя искусственное заражение массами яиц. Семь коммерческих гибрида и два гибридов **«opaque-2»** с открытым педигри, составлявших одну группу, имели на 17—26% больше мотыльков первого поколения и на 13% больше туннелей, чем нормальная гибридная группа. Четыре гибрида **«opaque-2»** с открытым педигри, входивших в одну группу, имели на 7% больше мотыльков первого поколения и на 16% больше туннелей, чем нормальная гибридная группа, и развитие мотыльков было на 0,11—0,28 стадий больше у гибридов **«opaque-2»**. Пять самоопылённых родителей **«opaque-2»** этих гибридов имели на 9% больше мотыльков первого поколения и на 21% больше туннелей, чем нормальные самоопылённые линии, и развитие мотылков было на 0,10 стадии больше, чем в самоопылённых линиях **«opaque-2»**. Степень самой большой чувствительности сортов **«opaque-2»** к первому поколению изменялась в зависимости от педигри.

Гибриды, в которых одним из родителей была линия B14A, показали «обратную реакцию», у них было на 6—15% меньше мотыльков в гибридной группе **«opaque-2»**. Однако развитие мотыльков было на 0,07—0,23 стадий выше в гибридах **«opaque-2»**. Четыре гибрида **«opaque-2»** с открытым педигри имели на 8—10% больше мотыльков первого поколения и на 16% больше туннелей, чем нормальная группа. **«Opaque-2»** и нормальные самоопылённые группы дали одинаковые результаты по заражению вторым поколением.

ЦИТОГЕНЕТИЧЕСКОЕ ВЛИЯНИЕ ХИМИЧЕСКИХ И ФИЗИЧЕСКИХ МУТАГЕНОВ НА РАЗВИВАЮЩИЕСЯ ЗАРОДЫШИ NIGELLA DAMASCENA L.

ФАН ФАЙ, В. Ш. АНДРЕЕВ

Разработан новый метод для обработки гамет, зигот и ранних зародышей **Nigella damascena L.** химическими и физическими мутагенами. Были обнаружены задержка в оплодотворении и замедление темпа клеточного деления в зародыше и эндосперме после мутагенного эффекта. Индуцирование хромосомных аберраций продолжается не менее 80 часов после химической мутагенной обработки, в то время как после облучения новые хромосомные аберрации не появлялись. Наблюдалась положительная корреляция между количеством хромосомных аберраций и понижением роста и развития растений. Частота клеток с хромосомными аберрациями, обнаруженных в анафазе 1 мейоза, достоверно повысилась после обработки мутагеном. В результате опыта количество стерильной пыльцы увеличилось и фертильность растений уменьшалась.

ДОКАЗАТЕЛЬСТВО ВЗАИМОДЕЙСТВИЯ СУБСТРАТА МИОЗИНА (АТП) С ГИСТИДИНАМИ И ДРУГИМИ ОСНОВНЫМИ АМИНОКИСЛОТАМИ С ПОМОЩЬЮ ФЛУОРЕСЦЕНЦИИ

И. КАША, Ш. ФАЗЕКАШ, В. СЕКЕШШИ-ХЕРМАНН, Е. ТИХАК

С помощью спектрофлуорометрии исследовались взаимодействие контактных аминокислот, играющих роль в ферментативной функции миозина АТП (Гис, Мегис, Лиз, Me$_3$ Лиз, Цис), и образование их келатов. Установлено, что Гис, МеГис, Me$_3$Лиз и Цис взаимодействуют с АТП и образуют келаты в присутствии Мг$^{2+}$, в то время как они вызывают значительное подавление флуоресценции АТП. Лиз не взаимодействует один с АТ и сдерживает подавление флуоресценции с помощью других аминокислот. В ходе взаимодействия АТП—Me$_3$Лиз, освобождается реактив фосфорил катиона, в результате чего фактически АДП взаимодействует с Me$_3$Лиз. Авторы предполагают, что фосфорил катион ограничивается МеГиз, и преимущественные гистидины миозина фосфорилируются через этот посредник. Важная роль приписывается дистеинам разной реактивности в связывании субстрата и особенно в наличии двух путей реакции.

ПОТРЕБНОСТЬ TRITICALE В ИЗВЕСТКОВАНИИ ПО СРАВНЕНИЮ С ДРУГИМИ ЗЕРНОВЫМИ

Л. М. МУГВИРА, К. И. ПАТЕЛ, П. В. РАО

Четыре почвы со значением pH ниже 5,0 были известкованы на уровне 0,0, 1,0, и 2,0 единиц, рекомендованных для пшеницы. Для сравнения, другая почва со значением pH 5,8 тоже получила известь, которая эквивалентна 0,0 и 7,3 мт/гектар. Три сорта гексапагоидного **Triticale**, одна пшеница и рожь выращивались в течение шести недель на известкованных почвах. В последующем эксперименте шесть различных сортов **Triticale**, пшеница, ячмень и два сорта ржи выращивались в течение восьми недель. Оба эксперимента проведены в растильных камерах. Применение нормы известкования, рекомандованной для пшеницы в Алабаме, повысило рост **Triticale** 6TA 131 и Траилблазер на двух почвах, 6TA 385 — на трёх почвах. Рожь Абрузи ни на одной из почв не имела эффекта, а сухое вещество пшеницы Артур повысилось на всех четырёх почвах, для которых известь была рекомендована. Известкование пятой почвы, имевшей первоначальное pH = 5,8 не повысило рост растений. В среднем по пяти почвам, известкование увеличило сухое вещество побегов пшеницы, **Triticale** и ржи соответственно на 87%, 51% и 20%. В известкованных почвах с высоким содержанием Al и Mn средний рост побегов разных сортов ячменя превышал рост растений на неизвесткованной почве в 2,0 раза, пшеницы — в 1,4 раза, **Triticale** — в 1,2 раза и ржи — в 1,0 раз. Тем не менее, повышая применение извести по сравнению с дозами, рекомендованными для пшеницы, в общем рост растений не повышался. Средние концентрации Ca, Mg, Al, Mn и P в **Triticale** были сходными со средними концентрациями в пшенице, но меньше, чем у ржи. Устойчивость к кислотности почвы была следующая: рожь > **Triticale** > пшеница > ячмень.

Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (3—4), pp. 253—262 (1976)

EFFECT OF AMINO ACIDS AND UREA-ADDUCT SUPPLEMENTS IN FATTENING FINISHING LAMBS

By

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In the course of fattening early weaned finishing lambs we studied the effect of dl-methionine and l-lysine added to the feed mixtures as well as the possibility of replacing the extracted soybean by an urea-adduct preparation (Research Institute for Vegetable Oils and Detergents, Budapest). The digestible crude protein content of the feed mixtures used was 12 per cent, and their starch value 72—75 kg/q. The experiments were carried out with meat-type merino hybrid lambs divided into 6 groups, each containing 12 ram and 12 ewe lambs. The best result was obtained with the group in which the soybean in the feed had been replaced by a super concentrate containing urea-adduct, and a supplement of 0.4 per cent dl-methionine and 0.3 per cent l-lysine had been added. This group showed a 13 per cent higher gain in weight and 6 per cent better feed conversion than the control. When the feed mixture containing soybean was supplemented by 0.3 per cent l-lysine, the gain was 8 per cent higher and the amount of feed mixture used for 1 kg weight gain 9 per cent lower. At the beginning and end of the experiment we took blood samples from the animals and determined — among others — the total protein, total amino acid, retained nitrogen, lysine and methionine contents of the plasma. The trend of the nitrogen content retained in the plasma — with the weight gain of animals taken into consideration — provides information about the incorporation of nitrogen, which is useful for the protein supply of the animal. The examination of the nitrogen retained in the plasma during the experiment is thus a good indicator of the trend of the nitrogen turnover. The lysine and methionine concentration of the plasma was not in accordance with the amount of synthetic amino acids added to the feed, unlike the results we have obtained with pigs.

Introduction

Recently, an increasing number of papers have been published on the amino acid demands of ruminants during the preruminant period as well, and on the efficiency of synthetic amino acids added to their feed. According to some authors, the amino acids absorbed from the digestive tract have a great influence on the metabolism and production of ruminants. In the case of ruminants, when supplementing the feeds with amino acids not only the amino acids necessary to build the host organism but also those required for the bacteria and microorganisms of the rumen must be taken into account. The authors agree that amino acid deficiency in calves and lambs mostly occurs at the time of weaning before rumination and supply data on the amounts of amino acid required for the growth of ruminants of different age as well as for their milk, wool and meat production. **Hogan** (1970) found the deficient protein and amino acid supply to be a growth-limiting factor in lambs. **Nimrick** (1971) presents a method of determining the amino acid requirement of growing lambs. According to **Hatfield** (1970), **Birdmoir** (1972), and **Zhebretzov-Vrakin** (1972) synthetic amino acids mixed with the feed of early weaned lambs have a favourable effect on the development, meat and wool production of the animals. **Carrico** *et al.* (1970), on the other hand, found the methionine supplement in the feed of sheep to be ineffective.

In order to clear up the uncertainties found in the literature concerning the amino acid demand of young ruminants as well as to solve the feeding problems of early weaned lambs we set up experiments. We added amino acids (dl-methionine and l-lysine or the mixture of the two) to the feed mixture of finishing lambs, and mixed urea-adduct or a super-concentrate containing it — partly by themselves, partly by using various amino acid supplements — and carried out experiments with the thus obtained feedstuffs.

The urea-adduct is made by a chemical procedure. The urea is processed with fatty acids of animal and plant origin, it is in this way that the so-called "urea-adduct" is obtained. The preparation has the advantage that the fatty acids serve as energy sources in the urea conversion, and the compound slows down and protects the hydrolysis of the urea so that it is not poisonous. According to experiments carried out at our Institute, with the urea-adduct a better conversion of urea could be attained in the ruminants, and its use ensured a more uniform mixing with the feed and a better granulation.

We set the aim of replacing the extracted soybean in the fattening feed mixtures of lambs by the mentioned materials applied at the proper ratio.

When determining the protein concentration of the feed mixtures we considered the results of **Hinds** *et al.* (1964), **Andrews—Ørskow** (1970), **Frederikson** *et al.* (1971), **Lawlor—Crowley** (1971) and **Huston—Shelton** (1971). In establishing the mixing ratio of the urea-adduct preparation we used the experimental results of **Ørskow** *et al.* (1971) and **Kakuk—Veress** (1970) and added 2 per cent pure urea (corresponding to a super-concentrate containing 8 per cent urea-adduct) to the feed.

Material and Method

Besides the data of weight gain and feed conversion, various biological parameters were also examined in evaluating the efficiency of the different diets. So the following determinations were made of the blood samples taken from the experimental animals:

1. the total protein content of the plasma according to the method of Phillips et al.;
2. the amount of rest nitrogen in the plasma by the titrimetric method of Rappaport—Eichorn;
3. the total amino acid content of the plasma by the Folin—Danielson method. (For the description of the three methods see **Bálint** 1962).
4. The concentrations of free amino acids in the plasma as well as the individual amino acids were determined by a BIOCAL 200-type automatic amino acid analyser. The isolation

from the plasma of free amino acids was carried out by the modified method of **Moor—Stein** (1954, 1958).

Before starting our experiments we carried out pilot lamb fattening in two series with amino acid- and urea-adduct supplements, respectively.

In the first series combing merino × Swedish landrace lambs weaned at the age of 80 days and kept in groups were used. Besides the experimental granulated feed mixture these animals were given hay ad libitum. As suggested by the results, lambs weaned at an advanced age do not satisfactorily utilize the amino acids, while the soya meal can safely be replaced by the super-concentrate containing urea-adduct.

In the second series the meat-type merino lambs kept in groups were given amino acids and super-concentrate containing urea-adduct, respectively, while sucking. In this pilot experiment series very good feed conversion results were obtained which, however, was not exclusively due to the feeds, because the animals, while kept together with their mothers, sucked them too, and were not entirely confined to the feed mixtures. On the basis of the equally favourable feed conversion values we have formed the opinion that feed mixtures given during the lactation period are not worth being supplemented with amino acids.

Utilizing the experiences obtained from the results of the mentioned two experiment series we carried on further investigations in the fattening plant of a co-operative farm (at

Table 1

Percentage composition of feed mixtures

Feed	Groups					
	1.	2.	3.	4.	5.	6.
Maize	36.6	45.0	44.0	38.1	43.5	40.3
Barley	38.0	39.6	40.1	36.0	40.4	34.0
Wheat bran	10.0	—	—	10.0	—	10.0
Lucerne meal	5.0	5.0	5.0	5.0	5.0	5.0
Extr. soya	8.0	—	—	8.0	—	8.0
AP 18	0.4	0.4	0.4	0.4	0.4	0.4
Feed lime	1.0	1.0	1.0	1.0	1.0	1.0
Feeding salt	0.5	0.5	0.5	0.5	0.5	0.5
XIX. unif. premix	0.5	0.5	0.5	0.5	0.5	0.5
Preparation containing 2 per cent urea	—	8.0	8.0	—	8.0	—
dl-methionine	—	—	0.5	0.5	0.4	—
l-lysine	—	—	—	—	0.3	0.3
	100.0	100.0	100.0	100.0	100.0	100.0
Crude protein %	14.07	16.95	16.66	14.28	17.15	13.96
Dig. crude prot. %	11.88	12.02	12.30	12.11	12.23	11.78
Lysine %	0.51	0.32	0.32	0.52	0.62	0.81
Methionine %	0.19	0.15	0.65	0.70	0.55	0.19
Starch value kg/q	72.63	75.47	75.05	72.30	74.87	72.55
Protein concentration %	16.36	15.93	16.39	16.75	16.33	16.24
Price of 1 q feed mixture Ft	306.68	304.78	373.50	375.40	448.99	395.91

Harkakötöny) with meat-type merino lambs weaned at the age of 35 days. The initial weight of the animals was 13.5 kg on the average, while at the end of the experiment 28.5 kg. The lambs were divided into 6 groups, each containing 12 ram and 12 ewe lambs.

The composition of the experimental feed mixtures and the results of their chemical analysis are shown in Table 1.

The digestible crude protein content of the feed mixtures was identical in all groups, with a slight difference in crude protein content and starch value due to the super-concentrate containing urea-adduct (urea supplementation). With the dl-methionine and l-lysine supplements only the amino acid composition of the feed mixtures changed.

The feed mixtures were available for the animals ad libitum; the hay ration was 200 g per animal a day.

During the experiment the change of weight of the lambs was checked by weighing every ten days.

In the course of the experiment blood was taken from the lambs on two occasions, first shortly after the beginning of the experiment, then immediately before its completion.

Results

The major indices of the fattening experiment carried out with early weaned lambs are summed up in Table 2.

The data of the table show that the experiment lasted for 65 days and by the age of three months the animals attained an average weight of 29 kg. The highest daily weight gain (239 g) was obtained with group 5, which exceeded by 13 per cent the weight gain of the control group. On account of the value of dispersion among the animals significant difference could not, however, be pointed out. Beside the urea-adduct these animals were given a

Table 2

Results of fattening experiments

		Groups					
		1.	2.	3.	4.	5.	6.
Initial weight	$\bar{x}$ kg	13.83	14.04	14.10	13.73	13.35	13.26
End weight	$\bar{x}$ kg	27.56	28.97	29.16	28.09	28.90	28.14
Total weight gain during the experiment	kg	13.73 ± 2.4	14.93 ± 1.4	15.06 ± 2.4	14.54 ± 2.2	15.55 ± 2.4	14.88 ± 2.0
	%	100	109	110	106	113	108
Number of experimental days		65	65	65	65	65	65
Daily gain in weight	g	211	230	232	224	239	229
	%	100	109	110	106	113	108
Feed mixture used for 1 kg gain in weight	kg	4.46	4.31	4.60	4.55	4.19	4.08
	%	100	97	103	102	94	91
Starch value	kg	3.24	3.25	3.45	3.29	3.14	2.96
hay	kg	0.95	0.92	1.09	1.04	0.95	0.93
Cost of feed mixture used for 1 kg weight gain	Ft	13.68	13.14	17.18	17.08	18.81	16.15

0.4 per cent dl-methionine and 0.3 per cent l-lysine supplement in the feed mixture. The feed mixture did not contain extracted soya meal, but with the amino acid supplements the price of the feed became higher than that of the feed mixed with soya. The gain of animals in Groups 2, 3 and 6 was but slightly behind that of Group 5 (230 g in Group 2, 232 g in Group 3 and 229 g in Group 6 a day). From the results we have drawn the conclusion that soya meal can be replaced by an urea-adduct preparation in the fattening mixture of finishing lambs. Gain in body weight attained with it was similar to that obtained when the extracted soya meal was supplemented with lysine (Group 6), or the urea-adduct with methionine (Group 3).

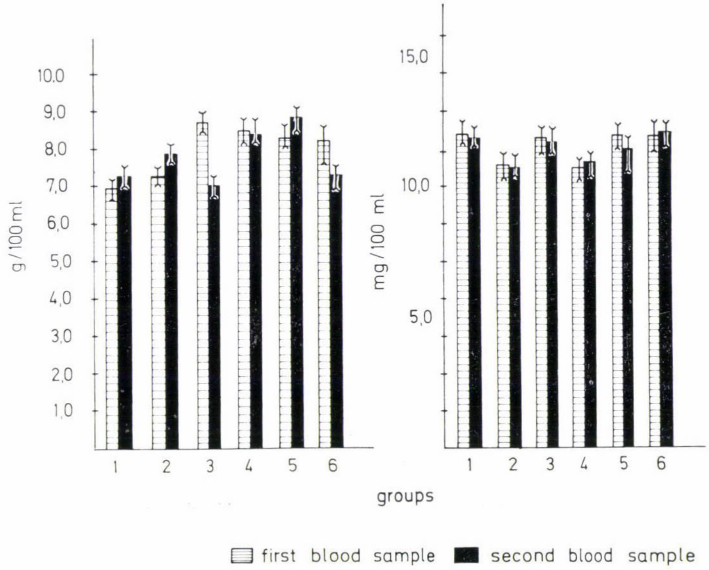

Fig. 1. Total protein content of the plasma. Total amino acid nitrogen content of the plasma

The amount of feed mixture used for 1 kg gain in weight was the smallest in Group 6, but when we consider the feed prices, the cost of feed mixture used for 1 kg weight gain was the most favourable in Group 2. Since the animals were kept in groups, dispersion and significance calculations cannot be carried out here. (Individual feed consumption data were not available.)

As to the total protein content of the plasma (Fig. 1) substantial differences between the groups could not be pointed out. On both occasions of blood sampling concentrations of 8.0—9.3 g/100 ml were found in the plasma.

In experiments carried out with sheep (Lewis 1957) and young fattening bulls (Juhász 1961) an adequate examination of the rest nitrogen and urea content of the plasma was found to provide reliable data on the nitrogen supply of the ruminants and the utilization of the nitrogen content of feed. The change in the amount of nitrogen retained in the plasma in the course of giving feeds of varying nitrogen content shows the extent to which the animals are able to utilize the nitrogen consumed with the feed and satisfy their nitrogen demands with it. In Fig. 2 we can see that the amount of rest nitrogen in the plasma was lower on the first than on the second occasion of blood taking. On the first occasion the amount of retained nitrogen was equal to that in the control Group in Group 4, lower in Groups 2 and 3, and slightly higher in Groups 5 and 6.

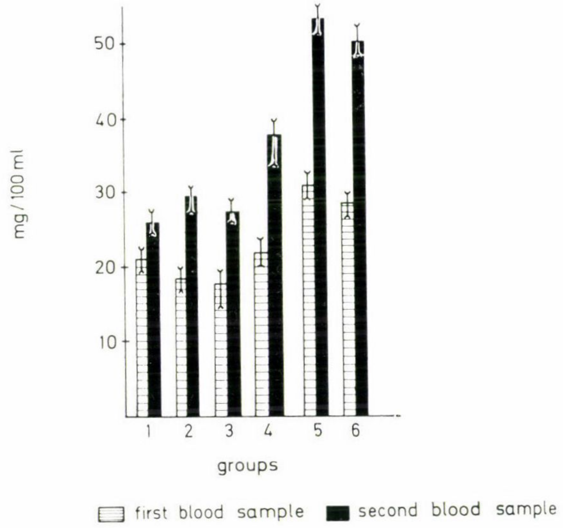

Fig. 2. Amount of rest nitrogen in the plasma

At the end of the experiment the rest nitrogen content of the plasma grew nearly identically in the control Groups 2 and 3, increased at a higher rate in Group 4, and rose substantially in Groups 5 and 6.

On the second occasion of blood taking the rest nitrogen content was in all groups significantly higher than on the first occasion. This suggests that the rest nitrogen content of the blood increases with age. On the first occasion of blood sampling its values ranged between 18 and 31, while on the second occasion between 26 and 53 mg/100 ml.

In our experiment the rest nitrogen content of the plasma was the highest on both occasions in those groups where the highest gain in weight and the best feed conversions were found. It was thus in the organism of animals in these groups that nitrogen consumed with the feed was best utilized. The lowest weight gain and highest amount of feed mixture used for 1 kg gain was found in Group 4; here the amount of nitrogen retained in the plasma, i.e. its rest nitrogen concentration was of medium value compared to the other experimental groups. It can be established from the experiments that generally those animals show the best gain in weight in which the rest nitrogen content of the plasma is the highest. A correlation can be pointed out — within certain limits — between the rest nitrogen content of the plasma and the weight gain of animals. A knowledge of the two values provides closer information concerning the proper nitrogen supply of young animals. E.g. the feed which while increasing the rest nitrogen content of the plasma does not result in a surplus gain — and vice versa — is not satisfactory. Further, Fig. 1 shows the total amino acid concentration of the plasma which did not essentially change during the whole experimental period; its average value was 11 mg/100 ml.

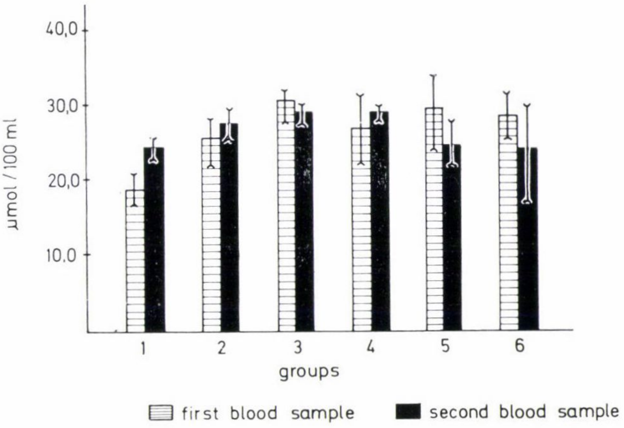

Fig. 3. Lysine content of the plasma

In Figs 3 and 4 the changes in the lysine and methionine concentrations of the plasma are shown. On the first occasion of blood taking the lysine concentration of the plasma in the animals of the control group (1) was lower (17.3 μmol/100 ml) than in those of Groups 3, 4, 5 and 6 (30.0 μmol/100 ml). On the second occasion of blood sampling, however, the results obtained in the experimental groups did not show any substantial difference compared to the control.

The methionine concentration of the plasma ranged between 2.3 and 4.3 μmol/100 ml during the whole period of the experiment, with the exception of the value of 1.8 μmol/100 ml obtained in group 6 on the second occasion of blood taking.

From these, and from the values seen in Fig. 2 we can draw the conclu-

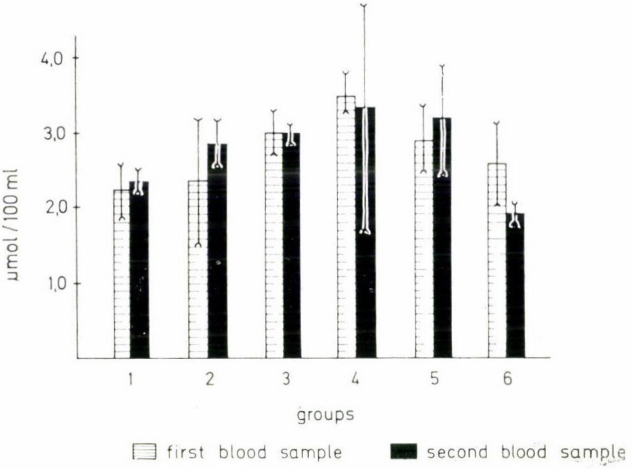

Fig. 4. Methionine content of the plasma

sion that in the course of fattening finishing lambs the nitrogen and amino acid contents of feed do not influence the amino acid concentration of the plasma.

Conclusions

According to the results of the experiments, lambs given an amino acid supplement attained a 6—13 per cent gain in weight compared to the control animals. Although their feed conversion also improved, still under the Hungarian conditions at the present synthetic amino acid prices, mixing either methionine or lysine with the feed for fattening lambs is not recommended.

Good results were obtained in lamb fattening by using the urea-adduct preparation of the Research Institute for Vegetable Oil and Detergents (Budapest). The parameters of weight gain, feed conversion and blood testing prove that the protein carrier import meals (soya, peanut, etc.) can be replaced in the feed mixture of lambs weaned at the age of 30—35 days by urea-adduct, because even these young animals are able to utilize the non-protein nitrogen (NPN) compounds. Last but not least, the use of the urea-adduct is justified by the fact that its price is much lower than that of soya.

Furthermore, in our experiment we wished to get information about the amino acid demand and optimum supply of finishing lambs. It is not, however, a simple task because the digestion of the rumen considerably influences the pathway of the amino acids consumed in the feed. In experiments carried out with goats Lazarov—Ivanov (1970) pointed out that the methionine and cistine were already absorbed through the wall of the rumen. Ørs-

KOW—FRASER (1969) as well as ØRSKOW—FRASER—CORSE (1970) and SCOTT—LITTLE—AMOS—MITCHELL (1972) also stated that there was an essential difference in the way of absorption of methionine applied orally or abomasally. Only the amino acids infused directly into the abomasum have an influence on the free amino acid content of the plasma.

On the basis of our experiments we can establish that if the feed mixture containing urea-adduct is supplemented with methionine and lysine the gain in weight and feed conversion of lambs substantially improve, while lysine when added to soybean slightly increases the gain but reduces the amount of feed required for 1 kg gain in body weight.

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Acta Agronomica Academiae Scientiarum Hungaricae Tomus 25 (3—4), pp. 263—268 (1976)

MACRO- AND MICROELEMENT CONCENTRATION IN SEEDS OF VEGETABLES BELONGING TO THE FAMILY CRUCIFERAE

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When examining 169 seed samples of vegetables belonging to the family of *Cruciferae* for 11 elements a high P/Cu ratio was found to be their most characteristic feature distinguishing them from all vegetables widely grown in Central-Europe. The ash component concentration of seeds was lower in tendency in varieties with a longer than in those with a short vegetative period.

Introduction

As a continuation of our earlier investigations (Tölgyesi 1968, Tölgyesi—Gellért 1971) we studied the seeds of major vegetables belonging to the family *Cruciferae*. They occupy 10—12 per cent of the total sowing area of vegetables in Hungary. The taxonomically closed uniform family has about 3000 species on the earth. They are fertilized by cross-pollination through the mediation of insects. Their seeds contain very little seed protein, their nutrient reserves are mostly stored in the form of oil in the cotyledons. Vegetable plants of higher practical importance within the family belong to two species: the radish varieties to ***Raphanus sativus*** L. and the varieties of cauliflower, cabbage (red cabbage), kale and kohlrabi to *Brassica oleracea* L. The knowledge of the compositon of elements in the seeds is an important theoretical and practical starting point. The seeds have a more permanent composition than the vegetative organs, and their macro- and microelement concentration ranges between narrower limits under the influence of external factors. The microelement concentration is phylogenetically determined in all plant organs, including the seeds, referring among others to their fertilizer requirements (Bowen 1966, Vlasyuk 1965) and to the ratio of their organic components (Tölgyesi 1969). It is useful to know the composition of the seeds as it gives a generally applicable indication concerning the soil and nutrient requirements and the use-value of the produced plant too.

Material and Method

We performed our examinations with 169 state-certified seed samples true to variety, obtained from specialized shops between 1964 and 1972. The seeds were measured out in an air-dry state, and determined for five macro- (K, Ca, P, Mg, Na) and six microelements (Fe, Mn, Zn, B, Cu, Mo) with the previously described colorimetric, flame-photometric and atom-absorption methods.

Table 1

Ash components in the seeds of cruciferous horticultural plants, with the mean values, standard deviation and variation coefficients indicated

		K	Ca	P	Mg	Na	Fe	Mn	Zn	B	Cu	Mo
		g/kg					mg/kg					
Red cabbage —	$\bar{x}$	5.94	5.09	10.19	2.98	0.083	137.9	41.8	40.6	22.5	3.46	0.25
Brassica oleracea L.	s	1.35	0.29	1.10	0.65	0.026	49.1	14.9	12.4	11.7	0.37	0.08
var. *capitata* f. *rubra* (11 pieces)	V %	22.8	5.75	10.8	21.6	31.1	35.6	35.8	30.8	52.0	10.8	32.0
Common white cabbage —	$\bar{x}$	6.81	8.65	10.9	7.79	0.135	119.7	37.6	37.3	21.3	3.91	0.44
Brassica oleracea L. var.	s	2.95	1.04	2.20	0.77	0.101	29.6	10.8	14.7	5.4	1.50	0.27
capitata f. alba (38 pieces)	V %	43.2	18.4	20.2	27.6	75.0	24.8	28.7	39.6	24.9	38.3	61.0
Kale — *Brassica oleracea* L. var.	$\bar{x}$	5.59	5.16	8.38	2.88	0.105	150.0	33.7	34.7	23.5	2.84	0.27
sabauda (36 pieces)	s	2.14	2.34	1.62	0.59	0.074	101.0	10.6	10.9	13.5	0.62	0.22
	V %	38.4	45.5	19.2	10.9	70.5	67.0	31.1	31.4	57.6	21.8	83.9
Kohlrabi — *Brassica oleraces* L. var.	$\bar{x}$	5.81	4.63	9.24	3.06	0.125	123.2	36.8	37.3	24.0	3.52	0.33
gongyloides (38 pieces)	s	2.20	1.34	2.23	0.56	0.125	45.7	13.1	12.6	7.0	0.93	0.21
	V %	37.9	29.0	24.2	18.2	100.0	37.2	35.8	33.8	29.0	26.4	63.7
Cauliflower — *Brassica oleracea* L. var.	$\bar{x}$	6.84	4.69	9.55	3.44	0.115	164.0	29.6	48.5	18.9	7.0	0.25
cauliflora (13 pieces)	s	3.22	1.21	1.82	0.26	0.129	70.7	5.73	25.6	8.5	15.8	0.14
	V %	48.4	25.8	19.3	7.7	113.0	43.0	19.3	53.0	45.5	226.0	56.0
Radish —	$\bar{x}$	5.96	3.35	8.16	2.93	0.118	167.0	24.0	40.0	19.7	4 59	0.37
Raphanus sativus L.	s	1.86	2.11	2.7	0.55	0.073	133.6	8.02	8.7	21.0	0.94	0.13
(33 pieces)	V %	31.2	66.4	29.4	18.8	62.5	80.0	33.4	21.9	107.0	20.5	37.3

Results

a) Specific composition of elements in the seeds of vegetables belonging to the Cruciferae. The examined seeds as a whole show a conspicuously low copper concentration (Table 1) which — with the exception of the cauliflower — significantly differs from the copper contents of all the other vegetable seeds. Their chemotaxonomic separation is even more striking if — taking their phosphorus content in consideration as well — we study their P/Cu ratio (Table 2). On the basis of the Dixon-test the outstanding P/Cu ratio of 2350 can be regarded as significant.

Table 2

P/Cu ratio of vegetable seeds as a chemotaxonomic means of isolating the cruciferous plants (Cruciferae)

Family	Number of examined		P/Cu
	species	samples	
Fabaceae	2	76	448
Umbelliferae	5	69	491
Solanaceae	3	129	472
Cruciferae	2	169	2350
Cucurbitaceae	5	73	495
Compositae	2	50	293
Chenopodiaceae	3	39	510
Polygenaceae	1	13	728
Liliaceae	5	46	710

Dixon-test for the P/Cu ratio of Cruciferae: 0.853, tabular value $P_{0,05} = 0.554$

Values similar to their high phosphorus concentration could only be measured in the seeds of the pumpkin, cucumber, sweet and water melon belonging to the family *Cucurbitaceae.*

The greatest difference in composition between the radish (*Raphanus, sativus* L.) and the cabbages (*Brassica oleracea* L.) is shown in the ratio of iron to manganese. The value of the Dixon-test for the 6.96 Fe/Mn ratio of the radish is, however, only 0.356 and attains a value of 0.612 with the exception of the cauliflower only. This is on the border of 5% (0.642) and 10% (0.557) probability of error. The radish seeds significantly differ from the seeds of cabbages also by the low calcium concentration. The Dixon-test is 0.553, the tabular values are $P_{0 \cdot 05}$: 0.560, $P_{0 \cdot 1}$: 0.482.

Among the examined species and varieties the calcium, phosphorus, sodium and molybdenum contents of cabbage, the manganese content of red cabbage and the boron content of kohlrabi were of the highest value. These values are, however, within the limits of a normal distribution, and on the basis of the Dixon-test do not differ from the other data. There is no outstanding value even among the lowest concentrations. These data have confirmed the chemotaxonomic uniformity of the plant group in question.

The variation coefficient characterizing the standard deviation of the samples is the lowest for the phosphorus and highest for the molybdenum. Of course, the errors involved in the analyses are also different for the two elements. In the case of phosphorus they are about 5% while for the molybdenum may even attain 20%. The variation coefficients of the samples — irrespective of the effects of habitat — suggest their plasticity, variability, i.e. their heterogenous nature. And these properties are highly important in the work of the plant breeder.

Table 3

Concentration of ash components in varieties with different vegetative periods

	K	Ca	P	Mg	Na	Fe	Mn	Zn	B	Cu	Mo
Common white cabbage, early (20)	7.9	5.8	9.3	2.9	0.133	131	43	41	21.2	3.98	0.46
Common white cabbage, medium late (8)	5.9	6.5	9.7	2.9	0.117	114	41	39	21.0	3.50	0.34
Common white cabbage, late (8)	4.9	6.1	8.4	2.9	0.109	89	36	29	23.4	2.75	0.29
Kale, early (13)	4.5	6.9	9.3	3.1	0.100	174	36	51	20.5	3.80	0.29
Kale, late (18)	6.2	4.1	7.7	2.8	0.112	166	29	31	26.0	2.90	0.27

b) Relationship between the vegetative period and the composition of elements. The complicated relationship between growth vigour, meteorological factors and nutrient requirements manifests itself in the length of the vegetative period too, which is characteristic of the variety. We tried to find out the correlation between the length of the vegetative period and the ash components of the seed. In the group of cabbages with a short vegetative period the varieties Dittmar, Júniusi óriás, Aranyföldi, Koppenhágai piaci and Szentesi-5 were studied; the medium late varieties were represented by the varieties Enckhuisen diadala, Braunschweigi and Pallagi, while those with a long vegetative period by Dán tartós, Amager, Csurgói and Hajdúsági. The early kale varieties examined were: Bécsi kapucinus, Vasfej, Szentesi korai-3, Adventy; the late ones: Vertus and Mohácsi. As seen in Table 3 the concentration of the ash components is generally lower in the late varieties. According to the *d*-test the joint samples of the early and medium late varieties did not significantly differ from the late cabbages. The significance of differences between early and late varieties could not be pointed out by the *t*-test either. In spite of this the tendency is considered as worth being mentioned, and we shall control its general validity by a comparison with the data of other plant species available.

We made calculations as to a possible relationship between the colour substances of the plants and the composition of elements in the seeds. The greatest difference was found in the Fe—Mn ratio of the white and blue kohlrabi (3.02 and 4.11 respectively). The difference between them is only tendency-like, as the calculated value of t is only 1.45 (tabular value of $t_{0.2} = 1.30$ of $t_{0.1} = 1.68$).

Discussion

Our investigations confirmed our earlier findings (TÖLGYESI 1965) and extended them to include the seeds of the vegetable plants too. Accordingly, in the more frequently studied 28 plant families the vegetative organs of *Cruciferae* have the lowest copper content (5.3 ppm) if we leave the gymnospermous pines (*Abietaceae* 5.2 ppm) and the monocotyledonous *Hydrocaritaceae* (5.3 ppm) out of consideration. Similarly low values were only obtained in the monocotyledonous grasses (*Gramineae:* 5.6 ppm Cu) and in the family of *Zosteraceae* (5.6 ppm Cu). So *Cruciferae* is one of the families containing the lowest amount of copper among the dicotyledonous plants. The typical and general nature of the high phosphorus content in the vegetative organs of cruciferous plants is proved also by the fact that they are in the second place — after *Solanaceae* — in this regard in the succession of the wild representatives of the mentioned 28 plant families. Extreme values concerning other elements were not revealed either by the earlier or by the recent investigations. All these data suggest that the definite concentrations of the inorganic substances are just as much the characteristic features of a plant as its other composition- or morphological properties are. As pointed out already (TÖLGYESI 1969), by the determination of the inorganic substances conclusions can be drawn on the taxonomic relationship, and from the taxonomic relationship — without analyses — on the concentration of the ash components. This fact is of invaluable importance in throwing light upon the processes of the nutrient chain, whether it is the soil requirement or fertilization, or even the question of feeding or nutrition in which we want to rely on general rules.

We wish to note that the other cruciferous species (white and black mustard, rape, turnip, field kale, garden cress, horse-radish, etc.) included in our material have the same properties. The composition of elements when known in the seeds or in the vegetative organs containing chlorophyll is suitable even in the case of 11 elements to separate taxonomic units, and — at the same time — indicates that the composition and the nutrient requirements are different in the plants. Many signs suggest that by studying further microelements we shall be able to know the biochemical habits of all cultivated plants and thereby promote the introduction of rational fertilization instead of an empiric nutrient supply.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (3–4), pp. 269–280 (1976)

THE VOLATILE OIL SECRETORY SYSTEM OF THE TARRAGON (ARTEMISIA DRACUNCULUS L.) LEAF

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The authors carried out histological and histochemical investigations into the ontogeny of the volatile oil secretory system of tarragon (*Artemisia dracunculus* L.) foliage leaves, and performed thin layer chromatographic studies too. They found no methylcavicol in the glandular hairs; the amount of this compound is related with the age of the leaf.

Introduction

Tarragon (*Artemisia dracunculus* L.) is in the first place a condiment of the preserves industry (GESSNER 1953), but is used also in the perfume trade and sometimes even as a medicine in the case of indigestion, inflammatory diseases or metabolic disorders, or as a diuretic, vermifuge and emmenagogue (BERGER 1954).

The plant has been studied at the Institute for a long time (TÉTÉNYI 1963, STIEBER *et al.* 1975).

The main active agent of the tarragon is the volatile oil produced partly in schizogenic volatile oil canals (Fig. 1), partly in characteristic *Compositae* glandular hairs (Fig. 2). Since the volatile oil composition of the glandular hairs and the schizogenic secretion canals is often different in the family — e.g. *Achillea millefolium* L., *Matricaria chamomilla* L. (HEGNAUER 1964) —, and according to some literary data glandular hairs are only found on young leaves, therefore their role in the composition of the distilled oil is not important either (ROSENTHAL 1954), we decided to take the question under closer examination.

Material and Method

Of the two types of tarragon: the so-called "French" or "German" (*Artemisia dracunculus* L.) and the so called "Russian" (*Artemisia dracunculus* L. var. *Redowskyi*) tarragon (TÉTÉNYI 1970) we used the new prospective variety "Zöldzamat" of the "French" tarragon in our experiments. It is a plant of fine morphological structure, 60—85 cm high, conic, erect, with an abundance of leaves. Its stem is firm, cylindrical, 5—7 mm thick in diameter, decompound, racemose (25—30 branches), of brownish green colour, glabrous, lignifying at the base. The leaf is linear-lanceolate with an entire blade and sharp apex, 3—10 cm in length and 3—10 mm in width. It is of medium or light green colour, shiny on both sides; when young, abun-

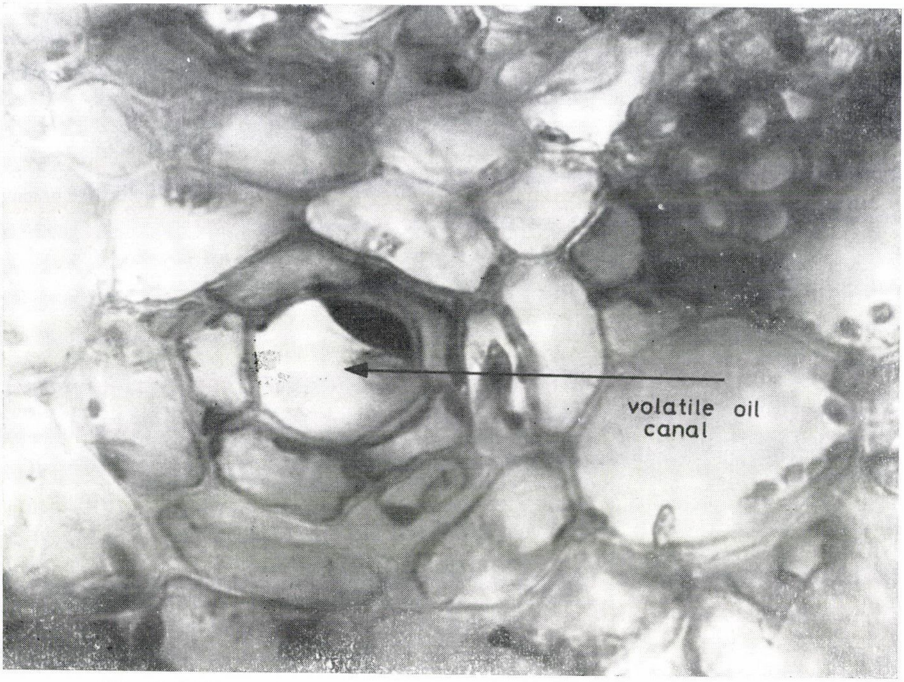

Fig. 1. Schizogenic volatile oil canal in a leaf cross-section (400 ×)

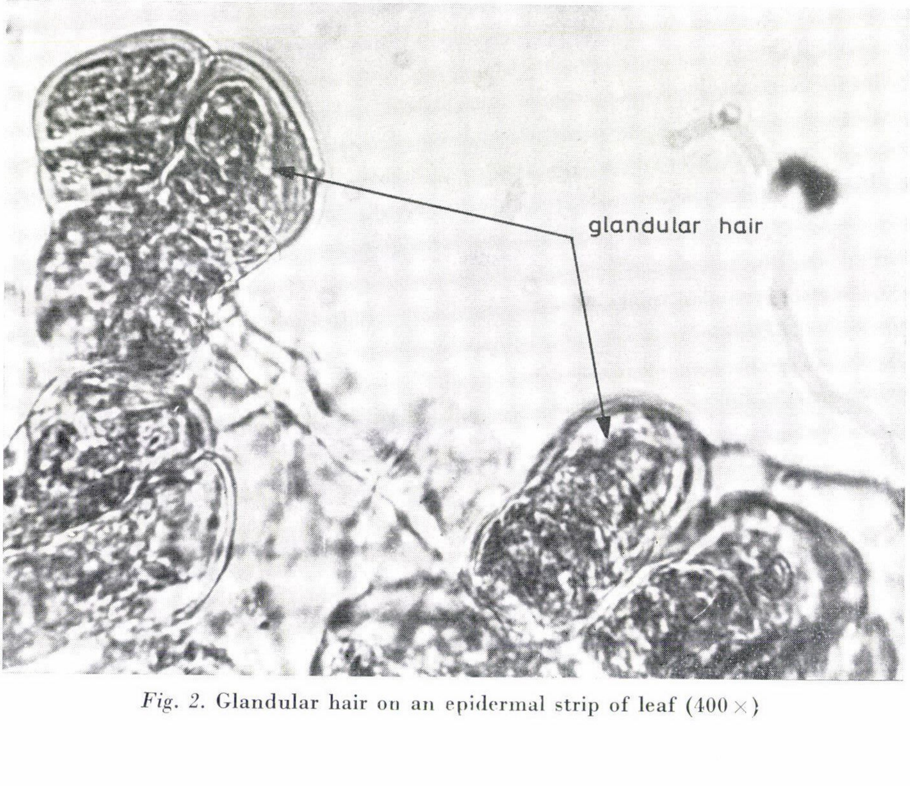

Fig. 2. Glandular hair on an epidermal strip of leaf (400 ×)

dantly supplied with glandular hair and thinly covered by bi- or trifurcate trichomes. Its root system is densely branching, lignifying, of greyish brown colour. From the buds of the rhizome many underground stolons of long internodes develop; they are of white or whitish brown colour, covered by bracts, and aboveground shoots grow out of them. The plants grow quickly, the stands are homogeneous and soon become closed (STIEBER *et al.*, 1975). Fig. 3 shows such a stand.

After vegetation had started samples were taken from the stand at two weeks average intervals: 10 full shoots on each occasion, cut immediately above the ground. The major morphological parameters were immediately measured and summarized in Table 1. Generally the 6th to 7th foliage leaves counting from the shoot apex were used for the examinations, but in the case of samples T_4 and T_8 all foliage leaves on the main axis were examined.

The investigations were made on three lines: histology, histochemistry and thin-layer chromatography.

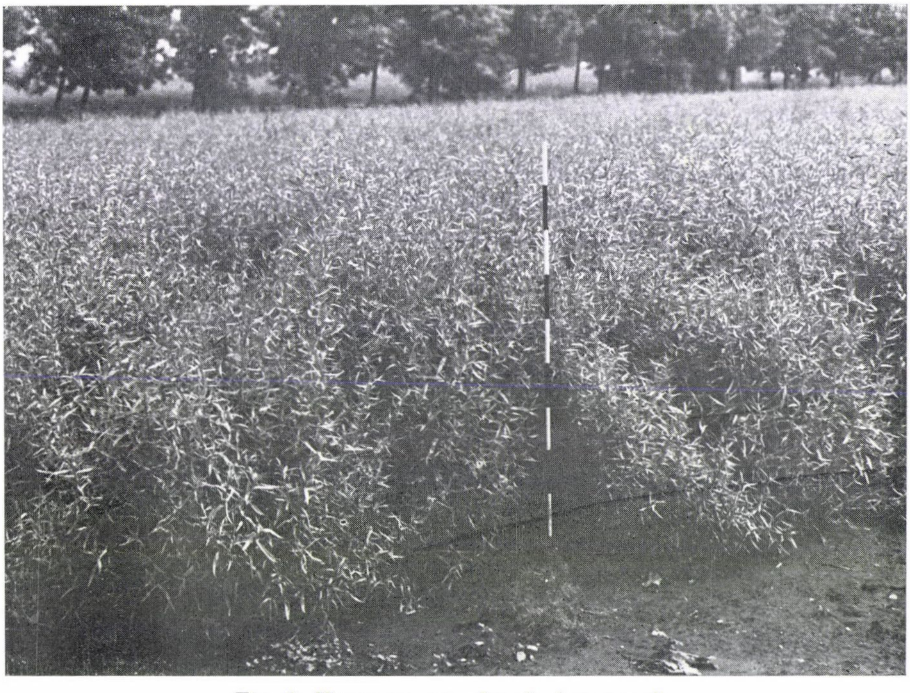

Fig. 3. Homogeneous, closed plant stand

a) *Histological examinations.* The histological studies were performed partly on epidermal strips, partly on sections prepared by freezing (Semiconductor microtome freezing stage TOC-II). The preparations were stained with a 0.2 per cent diluted solution of toluidine blue, mounted in aqueous glycerine and studied by a Reichert Zetopan microscope.

b) *Histochemical examinations.* 1. Reaction evoked by a concentrated hydrochloric solution containing 1 per cent vanillin. By means of a small distilling apparatus described by LUCKNER—BESSLER—LUCKNER (1966) the volatile oil was distilled into the reagent. As initial material we used either the 6th or 7th leaf counting from the apex, or an epidermal strip of the 6th or 7th leaf. In the latter case oil was distilled from the epidermis of three foliage leaves, while of whole foliage leaves only one was used.

2. The unstained preparations made according to *a)* were placed in Millon reagent, and an hour later examined.

3. Cytophotometric measuring. Preparations made by the *a)* method were treated with a 1 per cent diluted potassium permanganate solution for 2 minutes, then washed out in distilled water and mounted in aqueous glycerine. Measuring was carried out with the aid

Table I

Major morphological parameters

Sample		Plant			Stem		
		Height cm			Number of off-shoots		
Serial number	Time of sampling month, day	max.	aver.	min.	max.	aver.	min.
T_1	March 15	8	6	3	0	0	0
T_2	March 27	16	14	13	0	0	0
T_3	April 9	30	27	26	0	0	0
T_4	April 22	47	41	38	12	6	2
T_5	May 7	67	58	52	31	26	24
T_6	May 21	82	79	74	31	29	24
T_7	May 29	92	85	80	34	32	31
T_8	June 13	106	102	99	43	40	38

of a cytophotometric appliance of the Zetopan microscope (ob. 40×, oc. 6.3×, diaphragm 2.5). The glandular hair was measured in top-view at 410 nm; the same wave length was used in measuring various cells of a single glandular hair, too. Finally we measured the extinction of the same cell at various wave lengths.

c) *Thin-layer chromatography*. The layers were prepared in the usual way (STAHL 1967) with kieselgel G Merck, and activated over half an hour at 110 °C. The material was transferred on the layers by the TAS technique (STAHL 1969) at 140 °C. A mixture (96 : 4) of benzol-ethyl acetate was used as solvent. Distance between start and front was 12 cm. In the case of vanillin-sulphuric acid used as reagent, the slides were heated in an oven for 15 minutes at 110 °C after the evaporation of the solvent; if developed by R-Millon (THIEME—NGUYEN 1968) the spots were evaluated according to size after two hours of standing without heating.

Results

a) *Histological observations*. The histological observations were aimed at finding out how the hairiness of the foliage leaf changes until the first cutting. ROSENTHAL (1954) carried out detailed studies with tarragon and dealt — among others — with its hairiness too, trying to separate morphologically the "Russian" and "French" tarragon on this basis. In the "French" tarragon she found furcate trichomes only on the leaves of early spring shoots, while later, from the beginning of June the leaves were glabrous. At the time of cutting no trichomes were found.

As mentioned before, our experimental plant belongs to the morphological type of "French" tarragon. The furcate character is dominant in the trichomes (Figs 4—5). While in the case of young plants there are trichomes on the abaxial epidermis (T_1, T_2), in sample T_3 hardly any hairs are found from the apex to the 10—15th foliage leaf; from the 16th leaf downwards it is considerably hairy but here and there the places of broken-off hairs can be seen (Fig. 6). At the time of cutting, hairs can be found only sporadically on the lower leaves of the main axis. There are no trichomes on the foliage leaves *of the examined material*

Leaves											
Number of leaves per off-shoot			Number of leaves on the main shoot			Length mm			Width mm		
max.	aver.	min.	max.	aver.	min.	max.	aver.	min.	max.	aver.	min.
0	0	0	8	5	4	30	25	18	5	4	2
0	0	0	16	14	10	56	47	42	6	5	4
0	0	0	28	23	20	72	66	64	9	8	7
9	6	3	29	26	25	81	67	70	11	8	8
12	6	3	40	33	30	90	81	73	12	11	9
18	12	4	48	38	31	80	69	60	13	9	8
21	13	4	52	45	40	72	69	64	11	9	7
22	15	5	56	53	48	75	71	66	10	9	8

of the laterals. Our observations agree with those made by Rosenthal, only we found foliage leaves without trichomes developing at an earlier stage, this may be, however, a function of the weather.

The glandular hair is one characteristic of the *Compositae*, consisting of two rows of cells each containing three or four members (Fig. 2). On the

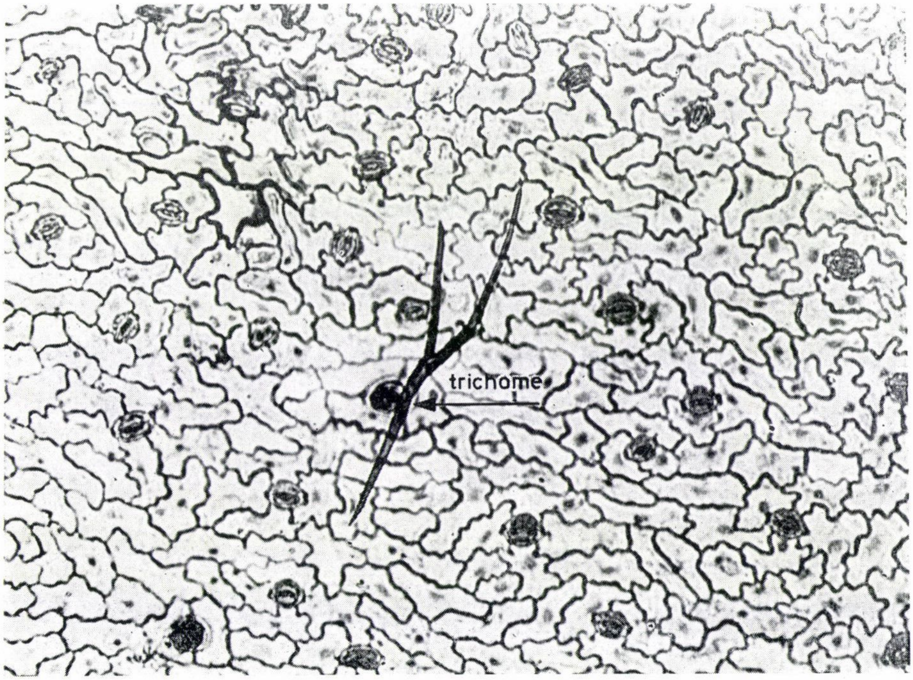

Fig. 4. Trichome on an abaxial epidermal strip of leaf (63 ×)

developing leaves these hairs are close to one another, later on the fully developed leaf they get farther, and some of them break off. The volatile oil produced lifts up the cuticle, therefore even the cuticles of the surrounding cells will be wrinkled. The stripiness of the cuticle can be seen at places where the glandular hair has broken off too (Fig. 7). If the cuticle bursts and the oil is exposed to the air often crystal needles can be observed. These crystals could be found not only in the stained preparations but also in the epidermal strips of the fresh foliage leaf mounted in water (Fig. 8). They have not been examined so far. When cutting we examined all leaves of a main shoot and found a lot of glandular hair on the 1—5th, less on the 6—8th, and hardly any on the 9—28th leaves (adaxial epidermis). On the abaxial epidermis glandular hair can be found only here and there on the 1—6th foliage leaves. Broken-off glandular hair cannot even be observed in traces. The young foliage leaves of the lateral shoots are covered by glandular hair. The distilled oil will thus contain the content of the glandular hair too, though its quantity is small in comparison with the amount of oil produced in the volatile oil canals of the of leaf and stem.

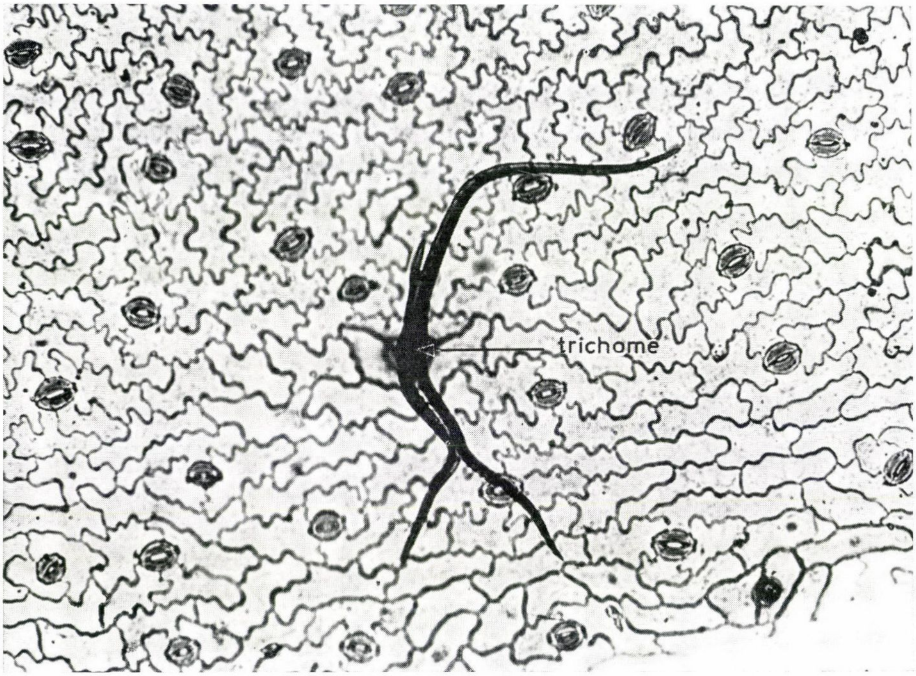

Fig. 5. Trichome on an abaxial epidermal strip of leaf (63 ×)

b) *Histochemical studies.* First we tried reactions which characteristically discoloured the distilled tarragon oil. One of these reagents was the concen-

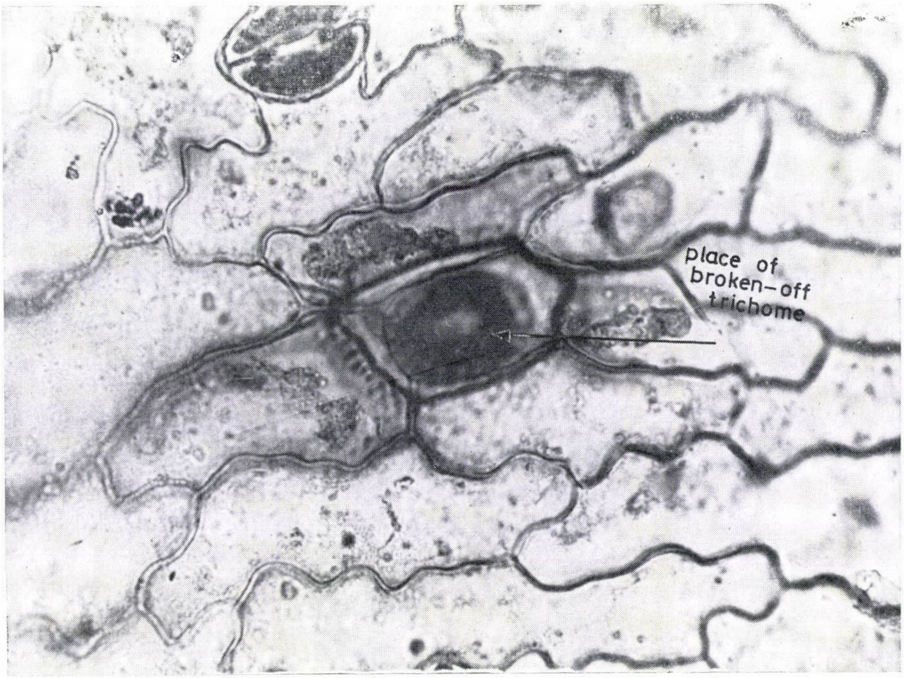

Fig. 6. Place of a broken-off trichome on an abaxial epidermal strip of leaf (250 ×)

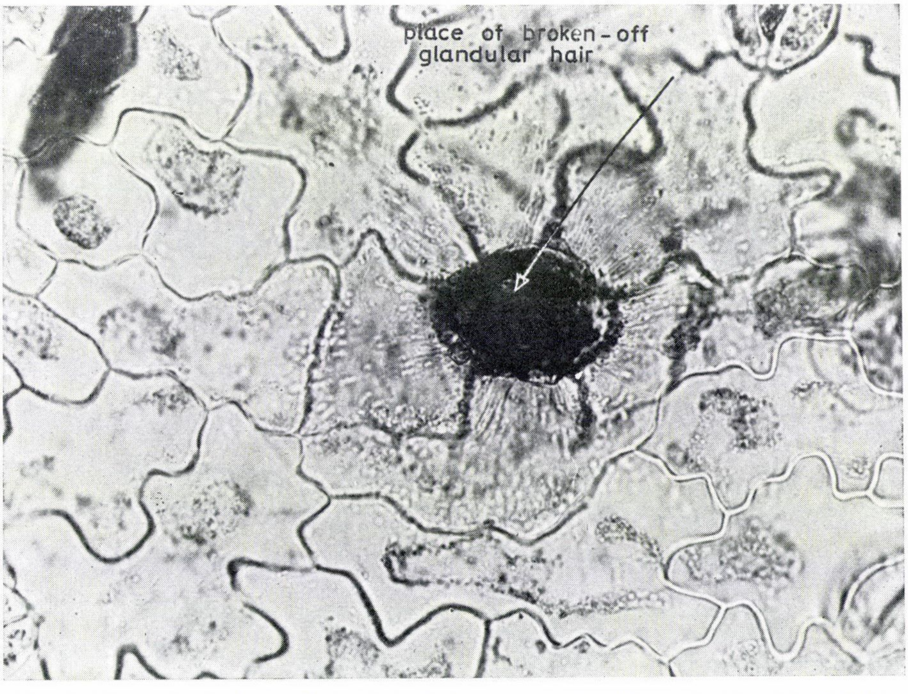

Fig. 7. Place of a broken-off glandular hair on abaxial epidermal strip of leaf (250 ×)

trated hydrochloric vanillin used by ROSENTHALER (1905) to identify volatile oils employed in large quantities, and by one of us (LASSÁNYI 1970) for identification in other plants. The distillate of the whole foliage leaf changed the colour of the reagent first into violescent pink, then — while standing — into dirty violet; when, on the other hand, the epidermal strip was distilled, the solution kept its original colour. Unfortunately, this reaction cannot be used for direct histochemical examinations because the concentrated hydrochloric acid destroys the tissues while standing.

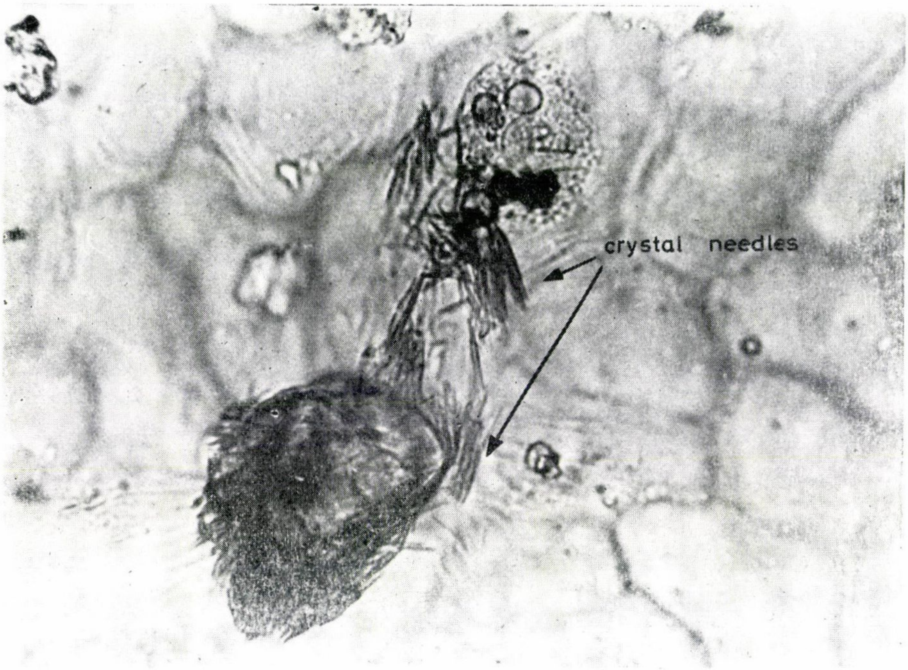

Fig. 8. Crystal needles of glandular hair (400 ×)

THIEME—NGUYEN (1968) used R-Millon for the spectrophotometric measuring of methyl-cavicol contained in the tarragon oil. The colour of the oil changed into red, and the developed colour was submitted to photometry after 2 hours. Since other phenols and phenolethers also have a part in the reaction, the reagent cannot be used directly, without separation except for the tarragon oil where methyl-cavicol is the main component (60—75 per cent). Considering that in the living plant the volatile oil is together with other components, our experiment was unsuccessful from the point of view of using the reagent for histochemical purposes, because not only the volatile oil canals and epithelial cells but also the contents of other cells and even the walls of the lignified cells changed colour.

We carried out cytophotometric measuring to characterize the glandular hair. The epidermal strips were treated with potassium permanganate. The permanganate can be used as a redoxindicator, because on the reducing effect of the immediate surroundings it changes its colour. With the identical treatment of various media it provides a possibility of semi-quantitative measuring too. It shows the following colours: with black reduction it changes to MnO_2, with brown to $Mn_3O_4(OH)_2$, with ochre to Mn_2O_3 (SZÉKELY 1963). Accordingly, the oxygen extraction is the lowest in the case of the ochre. Cytophotometric data obtained at various wave lengths are shown in Figs 9 and 10. The extinction values of all cells of a single glandular hair are contained in Table 2 while those obtained in top view of the glandular hair on three leaves of each of two identical samples are included in Table 3. As shown by a few selected data the cells have different reductive capacities. This difference can be found not only between the plants but also between the glandular hairs of a single foliage leaf and even between the cells of the same glandular hair.

Table 2

Extinction values at 410 nm of all cells of a single glandular hair

Sample	Serial numbers of cells*							
	1	2	3	4	4/a	3/a	2/a	1/a
T_3	0.35	0.36	0.31	0.23	0.40	0.42	0.41	0.34
T_6	0.40	0.56	0.50	0.40	0.31	0.50	0.40	0.31
T_8	0.36	0.40	0.33	—	0.36	0.51	0.45	—

* 1—4.: from the base of the glandular hair towards the apex
4/a—1/a: from the apex towards the base of the glandular hair

Table 3

Extinction values at 410 nm of glandular hair on 3 leaves of each of two plants

Sample	Leaves		
	1	2	3
T_8/a	0.25 0.60 0.68 0.47 0.05	1.20 0.80 0.32 0.19 0.61	0.48 0.36 0.40 0.28 0.32
T_8/b	0.40 0.61 0.30 0.82 0.72	0.66 0.48 0.60 0.90 0.66	0.29 0.76 0.34 0.66 0.60

c) *Thin-layer chromatography*. According to the literature (GUENTHER 1950, GILDEMEISTER—HOFFMANN 1961) methyl-cavicol is the main component of the tarragon oil, and almost the total amount of oil is supplied by the volatile oil canals, the volatile oil content of the glandular hair is negligible (ROSENTHAL 1954). The histological and histochemical observations were therefore supported by thin-layer chromatography too. We only wanted to prove the presence or absence of methyl-cavicol and did not pay attention to the other components. The boiling point of methyl-cavicol is 135 °C while that of most terpenes is around 220 °C, therefore we adjusted the heating to 140 °C according to the TAS procedure. In the course of the examinations

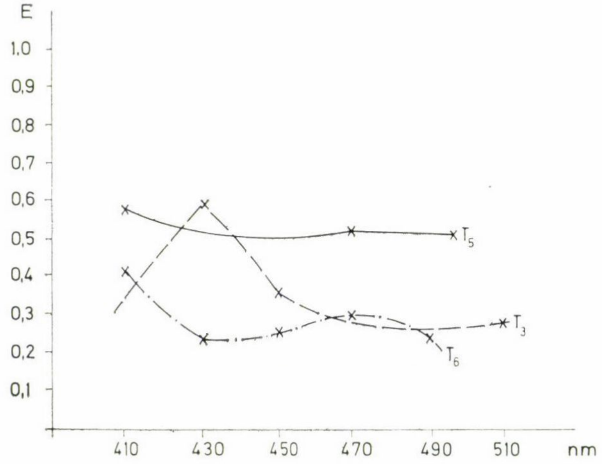

Fig. 9. Extinction values at various wave lengths of a single cell of each of samples T_3, T_5 and T_6

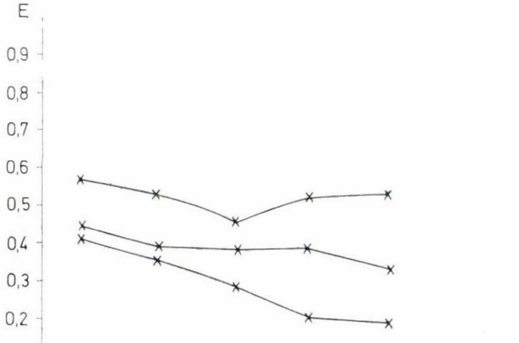

Fig. 10. Extinction values at various wave lengths of 3 cells in a single glandular hair of T_5

we found the methyl-cavicol content to be related with the age of the foliage leaves, and in this way with their position on the shoot, counting from the apex. The 1—5th foliage leaves contain methyl-cavicol only in traces; from the 6th leaf onward its quantity gradually increases to reach a maximum in the 11—23 foliage leaves, then decreases again. In the lowermost foliage leaves it occurs in traces, or totally disappears.

After development with vanillin-sulphuric acid a light yellow spot appears besides the lilac spot of the methyl-cavicol. By Millon reagent only a single red spot is obtained which agrees with the spot of the test. In the epidermis preparation methyl-cavicol could not be found even in traces.

On this basis our supposition of the volatile oil content of the glandular hair being different from that of the canals seems to be proved.

Conclusions

On the adaxial epidermis of the developing foliage leaf there is a thick cover of glandular hair; on the fully developed leaves it becomes thinner and from about the 15th foliage leaf counting from the apex the hairs break off. The abaxial epidermis of the young foliage leaves is thinly covered by glandular hair, later these hairs also break off. Covering hair is only found on leaves developing early in spring, but by the time of cutting even this disappears almost completely. Young foliage leaves on the laterals and the main axis are covered by glandular hair even at the time of cutting.

The secretion of the glandular hair and that of the volatile oil canals are not of the same composition. In the very young developing foliage leaves methyl-cavicol can only be found in traces. The largest quantities of methyl-cavicol are contained in the middle foliage leaves of the main axis, the amount of this compound decreases in the older leaves.

According to the cytophotometric measurements the glandular hairs of the same foliage leaf show different reductive properties (extinction), and even the cells of a single glandular hair do not give identical extinction values.

Acknowledgements

We are indebted to Miss Ágnes Györki and Mr. Gábor Varga for their assistance in the examinations, and to Mr. István Sipos for making the photos.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (3–4), pp. 281–289 (1976)

EFFECT OF FEEDING DIFFERENT LEVELS OF UREA ON THE NUTRIENT TURNOVER OF RUMINANTS

By

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In experiments carried out with Hungarian spotted bull calves and wethers the authors studied the effect of feeding three levels of urea on the apparent digestibility of the nutrient of feed rations and the nitrogen turnover of the animals. According to the results of their experiments the daily quantities of 20, 30 and 40 g urea per 100 kg live weight given to sheep, and 25, 37.5 and 50 g urea per 100 kg live weight fed to calves had not any considerable influence on the digestibility of nutrients and the nitrogen turnover of the experimental animals. On the basis of their experiments it seems to be reasonable to increase gradually the proportion of urea within the crude protein content of the feed rations. According to the authors' findings the proportion of urea in the feed rations of growing bull calves can also be increased without any decrease either in the digestibility of protein and other nutrients, or in the nitrogen retention.

Introduction

Due to the fundamental importance of the problem, investigations into the feeding of ruminants aimed at increasing the utilization of non-protein nitrogen compounds as protein sources are of interest both theoretically and practically. In Hungary many papers have been published on this subject during the last decades: Baintner (1956), Juhász (1962), Baintner–Bobek (1966), Barabás (1968). Nevertheless, first of all on economic considerations, it seemed to be advisable to study the nutrient turnover aspects of increased urea feeding in digestibility and nitrogen turnover experiments.

In the present practice the daily feed ration of ruminants generally contains 20–30 g urea per 100 kg live weight. In our investigations we wanted to find out what the effect of urea rations larger than the above on the digestibility of crude protein and retention of nitrogen was in the case of fully developed and growing animals — in sheep with nearly identical energy level but increasing amounts of crude protein, while in calves with increasing energy and crude protein levels but feed rations containing different quantities of urea. Furthermore, we wished to obtain data concerning the question whether in the case of giving non-synthetic feed rations urea added in large quantities to the basic feed would cause disturbances in the nutrient turnover.

The question of what the optimum amount of urea added to the feed rations of the ruminants as a protein source and favourably utilized by the animal organism is has been studied in a number of experiments. According to JOHNSON—HAMILTON—MITCHEL—ROBINSON (1942) 40 per cent of the crude protein content of a feed ration containing 10 per cent protein can be replaced by urea without any decrease in the nitrogen retention. DRORI—LOOSLI (1959) were of the opinion that if within the protein content of the feed ration the proportion of urea was over 40 per cent, the crude protein conversion was less favourable. FRENCH (1957) when evaluating the results of 134 digestibility experiments found no significant difference between the apparent digestibility of the nitrogen content of feed rations containing urea and the nitrogen digestibility of urea-free feed rations. It is naturally only true when the proportion of urea in the ration is not too high. In his opinion digestibility is in the first place a function of the total crude protein content in the ration.

In their sheep and bull fattening experiments DINNING—BRIGGS—GALLUP (1949) replaced 65 and 50 per cent, respectively, of the crude protein content of the daily feed ration by urea. They found in their investigations that it was not in the faeces but in the urine that the nitrogen content increased.

Beyond the quantity of urea in the protein content of the feed ration the utilization of urea is influenced by the composition of the basic feed too; urea is best utilized when combined with starch. The best utilization of urea can be attained by feeding maize then wheat and only then potato starch. SCHAADT—JOHNSON—MCCLUE (1966) increased the 6.5 per cent crude protein content of the basic feed to 12.3 per cent with urea. In their experiments of 72 days duration they arrived at the conclusion that with the transformation of the ruminal flora of ruminants, if the same nitrogen level feed ration was given, the nitrogen balance improved. BARTH—MCLAREN—ANDERSON—WELCH—SMITH (1959), and SMITH—DUNBAR—MCLAREN—ANDERSEN—WELCH (1960) published results similar to those outlined above.

Material and Method

The nutrient turnover experiments were carried out in the metabolism shed of the Research Institute for Animal Husbandry at Herceghalom with sheep and Hungarian spotted bull calves. The experiments were carried out with 3 wethers of 60 kg live weight per treatment, in four replications, and 4 bull calves of 200 kg live weight per group, in three replications, respectively. Thus, a total of 36 nutrient turnover trials were performed with sheep, and 36 with calves.

In the experiments carried out with sheep three urea levels were determined, where a daily amount of 20, 30 and 40 g urea per 100 kg live weight was fed in Groups I, II and III, respectively. In the experiments performed with bull calves three groups were included again; in Group I 25 g, in Group II 37.5 g and in Group III 50 g urea per 100 kg live weight was given. The animals were fed according to a feeding regime (Table 1). In addition to the basic feed the wethers were given a daily amount of 12, 18 and 24 g urea, respectively, evenly mixed with the fodder ration. Accordingly, assuming a complete conversion of the urea nitrogen 62, 69 and 74 per cent, while reckoning with a 50 per cent utilization 31, 34 and 37 per cent of the total crude protein was supplied as urea protein. In the feed ration of the bull calves

Table 1

Feeding regime

Group	Meadow hay	Maize meal	Barley meal	Feed starch	Molasses	Urea
	prescribed in the daily ration					
	g					
Nutrient turnover investigations made with wethers						
I.	600	200	—	—	50	12
II.	600	200	—	—	50	18
III.	600	200	—	—	50	24
Nutrient turnover investigations made with bull calves						
I.	2800	1900	300	—	—	50
II.	2800	1900	—	300	—	75
III.	2800	1400	—	800	—	100

the proportion of urea was 38, 57 and 63 per cent of the daily total crude protein in Groups I, II and III, respectively. We attained the required starch value and digestible crude protein level by changing the composition of the fodder and adding varying amounts of urea. The daily feed ration of the calves was supplemented with 50, 75 and 100 g urea in Groups I, II and III, respectively. The urea was evenly mixed with the feed and given to the calves twice a day. The prescribed feed rations were in every case consumed by the sheep to the full; the bull calves generally consumed the fodder and urea rations, but left some of the hay.

The animals were made accustomed to the prescribed feed rations during 14 days before the beginning of the nutrient turnover experiments, then a 7 days preliminary and 7 days experimental period followed.

In the technical implementation of the experiments, in the case of both the sheep and the bull calves, we followed the method of Mangold (1950), Wöhlbier—Eggers (1953), and Szücs—Molnár—Keresztes (1971).

The composition of the feed given to the animals, as well as of the feed remains and metabolic products was determined in each phase of the experiment according to standard. The starch value and digestible crude protein content of the consumed feed rations were calculated individually, with the nutrients' own apparent digestibility coefficients.

Results

The average feed and nutrient consumption of the animals as well as their feed conversion have been summarized in a table (Table 2).

The digestibility coefficients are also included in a table (Table 3). On the average of 12 sheep the apparent dry matter digestibility in Groups I, II and III was 54, 56 and 57 per cent, respectively. In the experiments carried out with bull calves the average dry matter digestibility was 62, 61 and 67 per cent in the same order of the groups.

Although a study on the organic matter digestibility never gives a full picture of the feed utilization, in many cases it may still provide an overall information. In the experiment carried out with sheep the apparent digestibility of the organic matters was 56, 59 and 59 per cent in Groups I, II and III, respectively. In the calf experiment, where the organic matter digestibility was examined as well, the figures were 65, 65 and 70 per cent.

Table 2

Trends of average feed and nutrient consumption, and of feed conversion in the experiments

Group	Daily feed consumption, g				Nutrient uptake			1 kg live weight increase attained with*	
	Concentrates	Meadow hay	Molasses	Urea	Starch value g	Digestible crude protein		starch value kg	digestible crude protein g
						g	%		
	Nutrient turnover investigations made with full-grown wethers								
I	200	600	50	12	320	57	18	—	—
II	200	600	50	18	387	77	20	—	—
III	200	600	50	24	383	95	25	—	—
	Nutrient turnover investigations made with bull calves								
I	2200	2538	—	50	2193	384	17	2.83	496
II	2186	2559	—	75	2187	386	18	3.83	676
III	2200	2772	—	100	2310	469	20	3.08	625

* Together with the requirements for maintenance

The apparent digestibility of the crude protein content of feeds shows larger differences compared to the former data. The crude protein digestibility as shown by the table does not fully agree with the statement of DRORI—LOOSLI (1959) that more than 10 per cent urea protein in the crude protein content impairs the conversion.

In the experiments performed with calves the protein digestibility was nearly identical — 63 and 64 per cent, respectively, — in Groups I and II, while in Group III — supposedly due to the higher rate of protein supply — it was 70 per cent. It is probably this that in the calf experiments explains the more favourable digestibility of crude fibre and nitrogen-free extracts — in addition to the organic matters — compared to the values obtained in Groups I and II. These results are supported by the data of AXELSSON (1938) and **Baintner** (1967) who found that a too small amount of protein decreased the digestibility, and optimum feed utilization only occurred in the case of a high protein content. In digestibility experiments carried out with feed of the feed ration increased relative to the dry matter content, utilization improved too.

Table 3

Trend of digestibility coefficients in the nutrient turnover experiments

Group	Dry matter	Organic matter	Crude protein	Crude fat	Crude fibre	Nitrogen-free extracts
	digestibility, %					
Experiments with sheep						
I. n = 12	54	56	64	54	45	60
S. D.	3.6	3.8	2.9	2.0	5.4	3.7
II. n = 12	56	59	71	56	46	61
S. D.	3.1	2.9	2.7	6.2	5.2	2.7
III. n = 12	57	59	73	52	44	58
S. D.	8.2	7.6	2.9	11.7	14.8	3.5
Experiments with bull calves						
I. n = 12	62	65	63	57	50	70
S. D.	1.8	2.0	2.4	10.1	3.5	2.6
II. n = 12	61	65	64	54	47	70
S. D.	3.8	3.4	4.6	10.0	9.7	4.4
III. n = 12	67	70	70	52	52	75
S. D.	3.7	3.3	4.6	8.0	11.0	3.2

The apparent digestibility of the crude fat content of the feed was 54, 56 and 52 per cent in the sheep experiments, and 57, 54 and 52 per cent in those performed with bull calves, in Groups I, II and III, respectively.

In the case of crude fibre all groups gave nearly identical results in the experiments carried out with sheep and in those performed with calves alike. In the experiments set up with sheep the average digestibility coefficients were: in Group I 45 per cent, in Group II 46 per cent and in Group III 44 per cent. According to the opinion of **Baintner** (1967) with the increase of the crude protein level the crude fibre utilization of the feed ration also increases compared to the deficient protein supply. No improvement of the apparent digestibility coefficients relative to the crude fibre was observed in this experiment. In the nutrient turnover experiments set up with bull calves the digestibility of crude fibre was 50, 47 and 52 per cent.

Studying the apparent digestibility of nitrogen-free extracts in the feed ration as a response to different urea levels, we obtained 60, 61 and 58 per cent digestibility in the sheep experiments, while in the calf experiments the digestibility of nitrogen-free extracts was 70 per cent in Groups I and II, and 75 per cent in Group III.

Table 4

Daily amount of nitrogen intake, excretion, digestion and retention in the experiments

Designation	Experiments with sheep			Experiments with bull calves		
	I	II	III	I	II	III
	group					
Number	12	12	12	12	12	12
Intake from the feed, g	14.32	17.39	20.27	97.79	97.08	106.78
Total excreted, g	12.13	13.95	16.22	54.15	46.74	60.04
of which						
in faeces	5.20	5.08	5.39	36.29	35.44	31.76
in urine	6.93	8.07	10.83	17.86	11.30	28.28
N digested, g	9.12	12.31	14.88	61.50	61.64	75.02
N retained, g	2.19	3.44	4.05	43.64	50.34	46.74
N digestibility, %	64	71	73	63	63	70
S. D.	2.9	2.7	2.9	2.4	4.6	4.6
N retention, %	15	20	20	45	52	44
S. D.	3.2	8.1	5.5	4.2	5.8	4.8

The apparent digestibility coefficients of the nutrients in the feed rations showed nearly identical tendencies in both the sheep and calf experiments. The difference between the digestibility coefficients was — in our opinion — due to the fact that the sheep were full-grown animals, while in the case of the calves developing organisms were involved. The digestibility coefficients may have been influenced by the different composition of the feed rations, too.

As for the standard deviation of the experimental data it can be established that in all groups of both the sheep and bull calf experiments nutrients present in small quantities in the feed ration show the widest standard deviation of the digestibility coefficients.

The average daily nitrogen turnover was also examined in the different urea level groups (Table 4). The data of the table show that in the case of sheep the increase of the protein level by urea improved the utilization of the nitrogen content materials of the feed ration. Of the daily average quantity of 14.32, 17.39 and 20.27 g nitrogen consumed in Groups I, II and III, respectively, the sheep digested 9.12, 12.31 and 14.88 g nitrogen and retained 2.19, 3.44 and 4.05 g nitrogen, respectively, which resulted in a 64, 71 and 73 per cent digestibility, and 15, 20 and 20 per cent nitrogen retention. As the sheep included in the experiment were full-grown animals, the effect of the daily nitrogen retention on the change of the animals' body weight was not examined. In the experiments carried out with bull calves the amount of nitrogen supplied a day was 97.79 g in Group I, 97.08 g in Group II and 106.78 g in Group III, on an average. The amount of nitrogen digested a day was in the same order of succession: 61.50, 61.64 and 75.02 g. In comparison with earlier investigations (Szücs—Molnár—Keresztes 1971) the retained quantity of nitrogen — 43.64 g in Group I, 50.34 g in Group II and 46.74 g in Group III on an average — was almost the same, but more than what had been expected.

Although according to our calculations the starch value and digestible crude protein content of the daily feed rations given to the calves was sufficient to increase their weight by about 750 g a day, the animals did not gain the weight expected on the basis of the daily amount of retained nitrogen (Group I: 775 g, Group II: 571 g, Group III: 750 g). Further investigations are required to detect the causes, although the individuality of the animals, on the one hand, and the stress caused by keeping them in the metabolism cage, on the other, may have played a role as well. The more favourable digestibility observed in Group III was supposedly the consequence of the better nitrogen supply. The percentage values of nitrogen digestibility and nitrogen retention show the following trend: 63, 63 and 70 per cent, and 45, 52 and 44 per cent, in Groups I, II and III, respectively.

The data obtained in the sheep and calf experiments seem to confirm the opinion of the literature — Dinning—Briggs—Gallup (1949), Hirose—Emery—Huffman—Conner (1960) and Farries—Zgajnar (1969) — that the amount of nitrogen excreted with the faeces hardly changes, while that in the urine increases with the increasing quantities of urea fed.

In the case of non-synthetic high urea content feed rations no disturbance in the nutrient turnover was observed either in the sheep or in the calf experiment.

Conclusions

On the basis of nutrient turnover investigations carried out with wethers and bull calves it seems probable that an increase of the daily urea ration from 20—30 to 40—50 g/100 kg live weight has no considerable influence on the digestibility of nutrients contained in the feed ration.

Replacement by urea of a part of the digestible crude protein content in the feed ration of ruminants does not decrease the nitrogen retention, if the proportion of urea in the protein content is gradually increased.

The proportion of urea in the feed ration can be increased in the case of developing organisms too, without any decrease in the digestibility of the urea protein and the other nutrients. According to the experimental results urea fed in increasing quantities did not adversely affect the incorporation of nitrogen.

On the basis of the nutrient turnover experiments it can be established that when urea is given in larger quantities the nitrogen content materials of the faeces do not increase, but the nitrogen content of the urine becomes somewhat higher.

A more extensive utilization of feeds containing non-protein nitrogen — first of all urea — in the feed rations of ruminants seems to be a reasonable idea.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (3—4), pp. 291—298 (1976)

HISTOCHEMICAL STUDY ON THE ENDOGENOUS CORKING OF JONATHAN APPLES

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The histological and histochemical aspects of corking occurring in Jonathan apples were studied. The affected tissue parts were tested for bacteria and fungi, respectively. The high diversity of the external morphological symptoms, the negative result of the microbiological test as well as the peculiarities of the histological characteristics prevented us from finding out the cause of the disorder.

Introduction

In some fruit-growing farms peculiar symptoms have been observed for the last three or four years. On the surfaces of the young small fruits sunken spots appear from the beginning of June. They are either bright red with occasional purplish discolouration, or remain green. Under the spots the flesh of the fruit is in each case corky. The corky region is found either immediately under the epidermis (discoloured surface) or deeper in the flesh (the surface remains green). At the time of fruit picking (end of September, beginning of October) the same symptoms can be observed on the normally developed fruit as well. However, trees producing such fruits show other symptoms too. Some fruits are deformed and/or have remained quite small. Beside spots with an average diameter of 3—6 mm substantially larger dents of various form — e.g. resembling the pressure of a branch — are found too. On the surface of some fruits several mm high protuberances of remarkable size develop. Palpable swelling lenticels are frequent; the fruit may be conspicuously firm with a colour differing from that characteristic of the variety (reminding of the varieties Staymared or McIntosh). Such trees occur in smaller or larger groups in the orchard, but perfectly healthy trees can also be found among them. On the other hand, trees whose fruits show the characteristic spots may also be found among those with symptomless fruits.

Material and Method

Fruit samples were obtained in June (1973) from the orchards of two farms (Balkány State Farm; "Petőfi" Co-operative Farm, Nyírmeggyes). Detailed analyses were performed with fruits picked on 12th and 26th June in the State Farm. At the time of the autumn fruit harvest (5th October) further fruit samples were taken in both orchards after a local survey for the purpose of histological and histochemical examination.

For the bacteriological investigations the friction preparations made from the corky spots and the adjacent tissues were stained with methylene blue (suitable to stain hyphae as well) or treated by modified Gram-staining. At the same time similar tissues were placed parallel onto bouillon culture medium, solid culture-agar plate and blood-agar plate containing 5 per cent bovine blood. Incubation was carried out at 37 °C over 20 hours in a thermostat, then for 24 hours at room temperature.

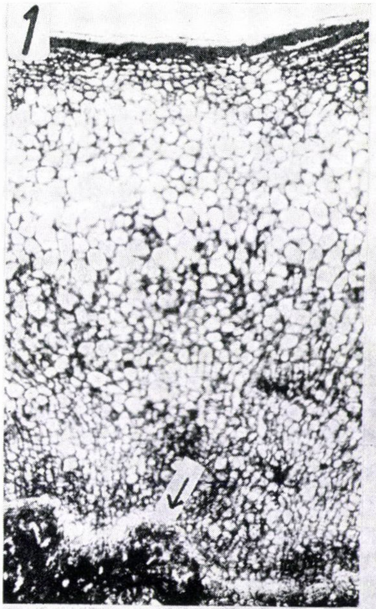
For the histological examinations the tissue parts excised from the fruit were fixed in a 6 : 1 : 1.3 ratio mixture of ethanol, formalin and picric acid, then embedded in histological paraffine with Halfacre's vacuum technique. The 12—15 micron thick sections were treated with a triple staining of Astrablau-auramin-safranin according to Maácz—Vágás (1961). In chemical control tests the staining technique was found to be of histochemical value. The age and differentiation of the cell-wall are indicated by the selective fixation intensity of the individual dyes. Some sections were treated with a universal histological staining (toluidin blue-safranin-eozin) after Csemniczky—Cziegler (1963). After both staining procedures Malinol (a synthetic mounting material) was used instead of Canada balsam. To identify suberification Sudan III. (Schneider 1922) proved to be suitable; after staining with Astrablau the slides were covered with glycerinegelatine.

Results

Bacteriological observations. The examination of preparations made from damaged tissues under the sunken spots or from the adjacent but apparently healthy flesh gave negative results. Hyphae or formations suggesting the presence of bacterium cells could not be pointed out by either of the staining methods. A similar result was obtained in the culturing experiments performed with various, otherwise highly reactive culture media, where no bacterial growth could be observed. Consequently, the possibility of a direct damaging effect by bacteria or other microorganisms detectable with the above methods had to be excluded.

Histological and histochemical examinations. The extent of various lesions or necrosis in the tissues under the sunken spots depends on how deep this area seen brown and corky with the unaided eye is located under the epidermis. The red or purplish red discolouration of the epidermis is one of the characteristic features of necrosis immediately under the epidermis. On the other hand, necrosis was unequivocally found to be general. That is, it uniformly affects the subepidermal collenchyme tissue and the adjoining parenchyma that forms the flesh of the fruit. On the contrary, the epidermis above a corky spot lying deeper in the flesh remains green, the collenchyme — though consisitng of more cell layers (Fig. 1) than in a healthy fruit — is not damaged. Again, Fig. 1 shows that the continued growth of the surrounding intact tissues results in a characteristic elongation of the adjoining parenchyma cells.

One of the most important characteristics of this deep-lying corky area is the necrosis of the tissues taking place in irregularly oriented, net-like zones (Fig. 2). On both sides of the zones consisting of collapsed cells a phellogenic activity starts, manifested in a rapid cell division with cell-walls parallel to the longitudinal direction of the zones. In fruit samples collected in June this type of cell division could be found in the necrotizing zones, but seldom



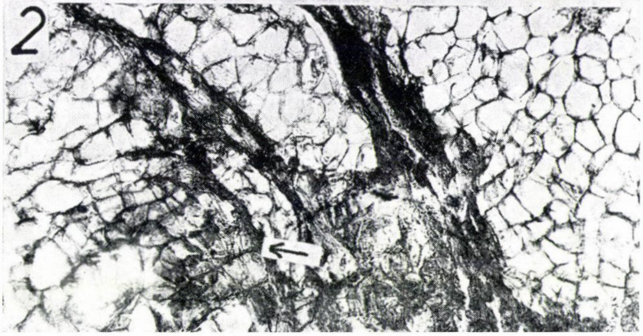


Fig. 1. Modified structure skin of the fruit above a corky focus (indicated by the arrow) developed in the parenchyma tissue

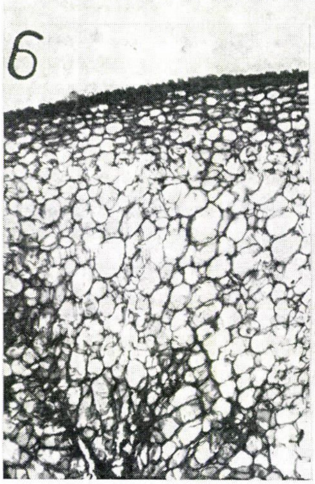
Fig. 2. Phellogen-like division zone surrounding the necrotic tissues

Fig. 3. Group of cells showing an irregular rapid division adjacent to the necrotic tissues

Fig. 4. Differentiating tracheids

— in any case not at a characteristic frequency — in the adjoining intact cells. On the other hand, in ripe fruits the occurrence of a zone representing the process of regeneration is common. The fast rate of cell division, and its realization at such an advanced stage is suggested by the fact that the cell-walls formed here often consist of pure cellulose (Astrablau staining). Again, it can be observed in many cases that the cell groups between the necrotized zones show an extremely high rate, but irregular, hyperplasia-like cell division (Fig. 3).

It deserves special attention that the "de novo" tracheid differentiation is not infrequent (Fig. 4). Quite young vascular bundles at the initial stage of



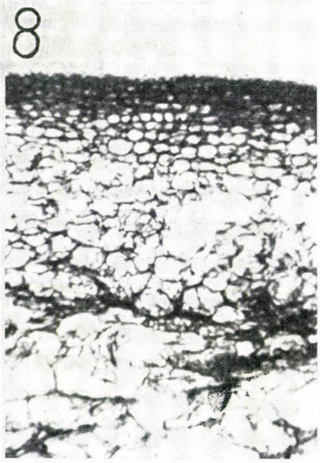


Fig. 5. Corky layer isolating the necrotic tissues from the normal parenchyma
Fig. 6. A section made from the protuberance appearing on the surface of the fruit
Fig. 7. Sclereids in the parenchyma
Fig. 8. Tissue structure of a hail mark

cell-wall thickening and only stained with Astrablau are easily found in the vicinity of necrobiotic spots. The simultaneous occurrence of corky tissue parts and vascular bundles with varying degrees of differentiation may be important for other reasons too. The corky tissue part is often joined with the unaffected tissues by beam-like converging bundles. In other cases the first symptoms of necrosis were observed in cells surrounding the vascular bundle. In spite of this we do not want to over-emphasize the possible role of the vascular bundles, if only because the fruit "tries" to isolate the region of net-like collapse with a phellogen-like zone of cell division (Figs 1 and 5), which results in a corky layer of intensive Sudan III staining.

The characteristics of suberification under the large dents of varying shape mentioned in the introduction agree with those of the corky area lying deeper under the typical sunken spots. However, the several mm high protuberances — strangely enough — are difficult to explain histologically. In the inside of the conic growths emerging from the surface of the fruit, corky tissues of the already known structure develop. The structure of the tissues surrounding the corky tissues does not, however, explain why a dent is not formed in this case above the necrotized tissue part (Fig. 6). Finally, in an interesting way, typical sclereids may occur in the damaged tissues or in their immediate vicinity (Fig. 7). Their presence is not characteristic of apples, we did not encounter them in our earlier investigations either.

For the sake of comparison we examined the marks caused by hail in the initial phase of fruit development (Fig. 8). The histological picture completely differs from the above and resembles the healing process of mechanical injuries described by PIENIAZEK—WISNIEWSKA (1971).

Discussion

The diversity of symptoms is one of the characteristic features of the above described damage of Jonathan apples. The different colour dents almost exclusively characteristic of the young fruit (picked in June) are the most frequent at the time of harvest too. The presence of spots on the otherwise not deformed, normal size fruit means — among others — that the spots, and the corky area below, may appear at any stage of the fruit development, in the same way as suberification caused by boron deficiency (BEYERS—TERBLANCHE 1971). We find no explanation for the symptoms of totally different nature occurring at harvesting time beside the typical 3—6 mm diameter sunken spots (see Results and Introduction).

It is an important point that nothing whatsoever suggests the disturbance of the vegetative growth of trees. In one of the farms (Balkány) the symptoms appeared on the fruits of 40 years old trees. The same trees produced 4—6 q fruit each and developed 40—60 cm long shoots. No symptom of leaves or irregular branching of the shoot system could be observed. Furthermore, the morphology of the lateral buds of long shoots and the well developed terminal buds of the so-called spurs promised abundant flowering for the next year.

It was a conspicuous phenomenon, again, that symptomless trees could be found in groups mostly consisting of diseased trees, on the other hand, trees showing symptoms on the fruit occurred in singles as well. Furthermore, the described symptoms do not necessarily extend to the whole crown of the tree, but may be restricted to fruits in a smaller or larger part of the crown.

Of the described histological observations two must be underlined. On the one hand, it is known that the apple grows by cell division only in the first phase of its development. Our first fruit sample originates from this phase of fruit development. In spite of this fact the regeneration of zones adjacent to the necrotizing tissue parts only appears sporadically and in no considerable extent. As contrasted with this, in ripe fruits, which under normal conditions are about to finish growing by cell elongation, there is an intensive activity of cell division (Figs 2, 3 and 5). Thus we have to reckon with the presence of a factor inducing the dedifferentiation of cells and retention of their ability to proliferate. On the other hand, it is remarkable that young cells formed after the division of the dedifferentiating parenchyma cells may differentiate into "de novo" tracheids (Fig. 4). In the vicinity of corky lesions or in living tissue parts between the necrotized zones the occurrence of young transporting elements showing cellulose staining and initial cell-wall thickening only, is not infrequent. It is worth mentioning that most of these symptoms show a pregnant similarity to those that have been observed by SIMONS *et al.* (1971) in relation to cork sport of York Imperial apple.

Trying to find the explanation of the symptoms we examined the probability of some possible causes. Boron deficiency was one of the suppositions. And indeed, boron deficiency causes a deformation of fruit, and spottedness in the inside of its flesh. Again, the absence of boron may result in a cellular proliferation, formation of brown substances in the cells, sclerosis of the fruit and protuberances on its surface (WIRTH *et al.* 1970). However, according to the authors just cited the spottedness is more or less uniformly distributed in the cross-section of the fruit which cannot be confirmed in our case. Further, the deficiency symptoms appear first in the growing parts (NAGY 1965), and the developmental disorders of the vegetative organs, in general, are always described as concomitant signs (WALLACE 1961, WIRTH *et al.* 1970) in the case of both other fruit species (KIM *et al.* 1969, JACOB *et al.* 1972) and vegetable plants (WETZOLD 1972). Such symptoms were not encountered in the orchards studied by us. It must be noted, however, that the fruit symptoms of a boron deficiency may appear without the disorders of the vegetative development (BEYERS—TERBLANCHE 1971).

Boron deficiency is generally accompanied by an imperfect fruit setting. The application of boron may therefore enhance the setting of apples (DAVISON 1971); when supplied by spraying it increases the productivity of pineapple (SHRIVASTOVA 1970) and may decrease the fruit drop of seed-producing ornamental trees (NEKRASOV—KNYAZEVA 1971). As pointed out above, in one of the farms (Balkány) the trees gave a maximum yield, fruit setting was thus satisfactory. The leading expert of the other farm (Nyírmeggyes) estimated the fruit drop observed in summer from trees showing the characteristic symptoms at 5 per cent. This extent of fruit drop may also be the result of often drastic disorders of fruit development. It seems to contradict the supposition concerning the boron deficiency that in the visited orchards the appearance of the symptoms was independent of the topographic conditions, and even of the earlier soil melioration. On the other hand, the above mentioned joint occurrence of healthy and diseased trees, as well as the often heterogenous distribution of damaged fruits within the crown make the trace element deficiency questionable. It is a serious counter-argument that in the orchard at Nyírmeggyes regular spraying with Wuxal (a complex nutrient solution containing boron) was carried on both in the year of the investigation and in that preceding it.

In any case the possible role of boron in producing these symptoms can be controlled in the next vegetation period, either by assessing the effect of a boron application started in due time (Wirth *et al.* 1970, Davison 1971), or by examining whether the boron content of leaves attains the critical level (Kim *et al.* 1969, Beyers—Terblanche 1971, Yamazaki *et al.* 1971). Finally we mention here that symptoms similar to those caused by potassium, boron and iron deficiencies may be produced by temperature conditions or viruses as well (Refatti 1971).

We will not dwell on other possible causes of the symptome described. The idea that they are caused by frost damage is disproved by the fact that the development of the mentioned symptoms is protracted in time, and that the symptoms are diversified and peculiar. Furhermore, histological disorders described above are quite different from frost injury in fruit tissue (Simons *et al.* 1970). Other disorders frequently occurring on the Jonathan apples (Jonathan spot, bitter spots, damage done by hail etc.) are totally different in appearance from those discussed here. The tissue structure of the Jonathan spot was described by Krapf (1961), and the bitter pit by Simons (1962), while the bitter spots, hail marks and lesions caused by scab were not found identical with the above symptoms in our own histological control examinations.

According to one of the authors (J. M.) Ca deficiency increased by an overdose of NH_4-nitrogen may induce the phenomena described by us. Indeed, Shear—Faust (1971) reported on a considerable reduction of the Ca level of fruit in case ammonium nitrogen was available for the roots in the period of calcium uptake by the fruit. At the same time, most of the various spots occurring on the fruit can be prevented, or at least reduced by calcium treatment.

Acknowledgements

We are indebted to Dr. Sándor Bodnár, head physician for performing the bacteriological examinations and to Mrs Gabriella Bodnár, biologist, for her assistance in making the microscopic photos (Public Health and Epidemiological Station, Nyíregyháza). Thanks are due to Miss Ilona Tóth, laboratory assistant, for carrying out the microtechnical work.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (3—4), pp. 299—308 (1976)

STUDY OF FREE AMINO ACIDS OF GRAPE BERRIES ON FIXION 50 × 8 LAYER CONTAINING CATION EXCHANGING RESIN

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The free amino acid composition of the berries of three grape varieties (*Vitis vinifera* L., cultivar Rizlingszilváni Syn. Müller Thurgau, Olasz rizling Syn. Wälschrieling, Piros tramini Syn. Gewürztraminer) was studied by chromatographic separation on a Fixion 50×8 type resin-coated chromatoplate containing cation exchanging resin. An analytical technique was developed by which the one-dimensional separation of about 16—17 amino acids from the grape berry can be carried out, rendering possible the rapid comparison of a number of samples in the case of serial examinations. Comparative studies were performed on plants grown outdoors and in culture pots, as well as on control and nitrogen-treated plants, with regard to the change of the free amino acid composition of the berry as a function of the course of ripening. In the sap of the grape berry proline and arginine accumulated in the largest quantities during the ripening and in response to nitrogen treatment.

Introduction

A number of publications have recently been published, mainly in the foreign literature, with regard to the chromatographic examination of free amino acids in grapes. A considerable part of the investigations concerns the role played by the free amino acids of the grape berry and grape juice in the metabolic processes, and their relationships with the ecological and economical factors and the phases of vegetation.

Fruits are known to be very poor in amino acids as compared to vegetables. Of the fruits, the grape and the tropical fruits contain the largest amounts of amino acids (Silber—Becker 1960). Similar results were obtained by Lindner in the qualitative and quantitative analyses of free amino acids in Hungarian vegetables and fruits. Papers published by Kliewer—Nassar—Olmo (1966) and Kliewer (1967, 1968, 1969) give an overall survey of the free amino acids in more than 20 varieties of the genus Vitis. They studied the qualitative and quantitative changes of the free amino acids of grapes during ripening. They mention 8 amino acids as main components: alanine, γ-aminobutyric acid, arginine, aspartic acid, glutamic acid, proline, serine and threonine. In the ripe fruit proline and arginine were found in the largest quantities, as previously pointed out by Lafon—Lafourcade—Guimberteau (1962), who studied the course of ripening in the grape varieties Merlot and Cabernet-Sauvignon. Similar results were obtained by HACHIDZE—MATIKASHVILI (1973) in examinations of leaves of various vine varieties, MARUTJAN (1966) examined early and late grape varieties for changes in their free amino acid composition.

KLIEWER—OUGH (1970) studied the effects of leaf area and yield amount and KLIEWER (1971) that of nitrate treatment on the free amino acid composition of grapes and found linear correlations between the amount of arginine and the leaf area per plant, and the nitrate content of the applied solution, respectively.

Of the examination methods for amino acids chromatography has proved to be the best. Identification of the free amino acids of grapes has mostly been carried out by the classical methods of one and two directional paper chromatographic separation.

The introduction of the amino acid analyser improved the methods, but since the instrument is expensive and its application requires much time and work it is not frequently used in serial examinations.

It was therefore necessary to elaborate a separation method which ensured an easy and quick identification, and in serial examinations gave an overall picture of the sample and made it possible to separate a large number of components so that the obtained components could be evaluated quantitatively too, either visually or instrumentally.

Material and Method

The aims of the present work were to examine the free amino acid composition of the berries of certain grape varieties and to test the applicability of the up-to-date chromatographic method necessary for the qualitative and quantitative analysis of the predominant amino acids.

For the examinations a resin-coated chromatoplate was used, containing a strong cation exchanging resin marketed under the name Fixion 50 × 8. This is a Hungarian product elaborated for practice by DÉVÉNYI—ZOLTÁN (1970). This layer contains Dowex 50 × 8 cation exchanging resin on plastic film. A detailed account has already been given (DÉVÉNYI 1972) of the advantages of the Fixion layer as compared to the classical layer. Here we single out only the following:

1. The chromatographic behaviour on the resin should be interpreted as the resultant of the joint effects of the adsorption, distribution and ion exchange conditions.
2. Owing to its excellent resolving power, one-dimensional separation of 16 amino acids is made possible.

The material used in the experiments was collected in the Szigetcsép Experimental Station of the University of Horticulture from own-rooted vines planted in 1967. Samples were taken on seven occasions during the vegetation period between 17 July and 15 October 1973.

The change of the amino acid composition in response to increasing amounts of nitrogen applied with a simultaneous medium rate phosphorus and potassium fertilization was studied in the berries of the variety Olasz rizling grown outdoors and in culture pots and on the variety Rizlingszilváni grown outdoors (KOZMA—POLYÁK 1973).

An average sample consisting of some 150 berries per treatment was collected. The berries were pressed with a fruit centrifuge, and then the material was purified by continued centrifuging (at 8000 r/sec). The juice thus prepared was freed from protein by precipitation with ethanol at a final concentration of 80 per cent, then left to stand for 12 hours at a temperature of 0 °C. Carbohydrate was removed according to **Kliewer** (1967a) by washing the material with distilled water through a Dowex 50 × 8 cation exchanging resin column. The amino acids were eluted with 3 N ammonium hydroxide.

The eluate was distilled under atmospheric pressure at 80 °C, and the residue dissolved in 10 per cent isopropanol.

For purposes of identification the prepared samples were applied to the Fixion layer by a calibrated capillary tube together with a universal standard solution consisting of 16 amino acids. The standard amino acids were also dissolved in 10 per cent isopropanol. Development was carried out with 0.4 M Na citrate buffer of 3.3 pH according to the method described by **Dévényi** (1971). The plates were developed partly with acetone ninhydrin (**Tyihák** 1966), and partly with the Moffat—Lytle polychromatic ninhydrin reagent modified by **Krauss—Reinbothe** (1970). The spots that appeared following the ninhydrin reaction were evaluated visually on the basis of their Rf values and colours, and semiquantitatively with a Chromoscan densimeter. With a view to a subsequent comparative study and quantitative evaluation, material was also prepared for measurement with the amino acid analyser.

Results

Fig. 1 shows the distribution of the standard amino acids and those of the grape sample on the Fixion 50 × 8 type resin-coated chromatoplate.

The amino acids identified on the layer and also proved to be present by the amino acid analyser were: Arg, Lys, His, Tyr, Leu, Ile, Val, Pro, Ala, Gly, Glu, (Ser-Thr) and Asp.

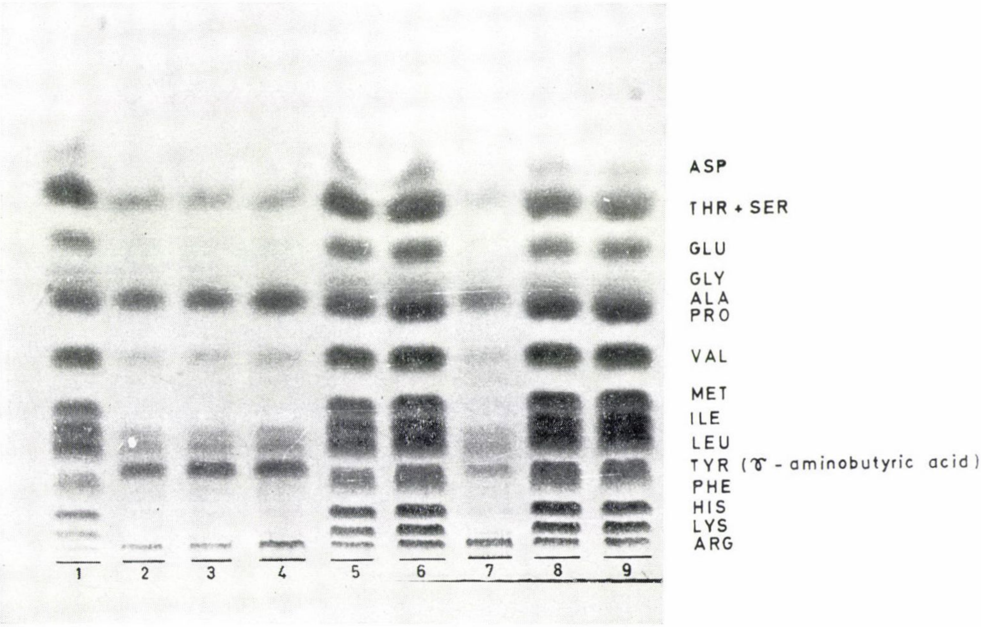

Fig. 1. Separation of free amino acids of grape samples harvested on 15 October 1973 on Fixion 50 × 8 cation exchange layer. (1), (5), (6), (8), (9): standard amino acid mixtures containing 5, 7, 10, 12, 15 μg per amino acid; (2), (3), (4): Rizlingszilváni control, nitrogen and potassium treatments; (7): Olaszrizling. Of the samples 40 μl juice was applied. Development with collidine ninhydrin (45 °C)

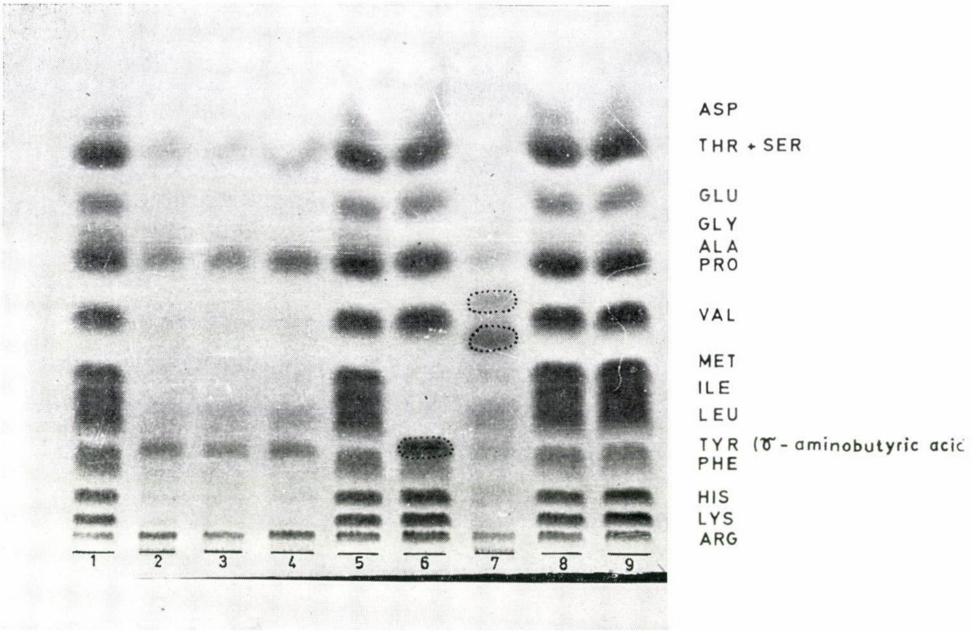

Fig. 2. Separation of free amino acids of grape samples harvested on 19 September 1973 on Fixion 50 × 8 cation exchange layer. (1), (5), (8), (9): standard amino acid mixtures containing 5, 7, 10, 12 μg per amino acid; (6): standard mixture containing γ-aminobutyric acid (10 μg); (2), (3), (4): Rizlingszilváni control, nitrogen and potassium treatments (40 μl each); (7): Olaszrizling (40 μl). Development with cillidine ninhydrin (45 °C)

Besides the amino acids indicated by the amino acid analyser a very intensive ninhydrin positive spot appeared on the layer in the September–October samples with a value (Rf-0.15) more or less corresponding to the Rf value of Tyr; according to the identification made by us on another layer and in a different running system, this was γ-aminobutyric acid (Fig. 2).

In addition to this we found phenylalanine at a low concentration; the amino acid present with an Rf value lower than that of valine was assumed to be norvaline.

When the composition of the reagent and the development temperature are altered, the amino acids are developed in different qualities and quantities. On the chromatogram shown in Fig. 3, which was developed with collidine ninhydrin at 110 °C, fewer amino acids are seen, but the reagent is strikingly sensitive to proline, γ-aminobutyric acid and arginine.

Figs 3, 4 and 5 show the changes of concentration of arginine, γ-amino butyric acid and proline in samples taken at different dates.

Excessive accumulation of proline in the period after ripening, especially in the variety Rizlingszilváni and mainly in the case of nitrogen and potassium treatments, can be observed even visually.

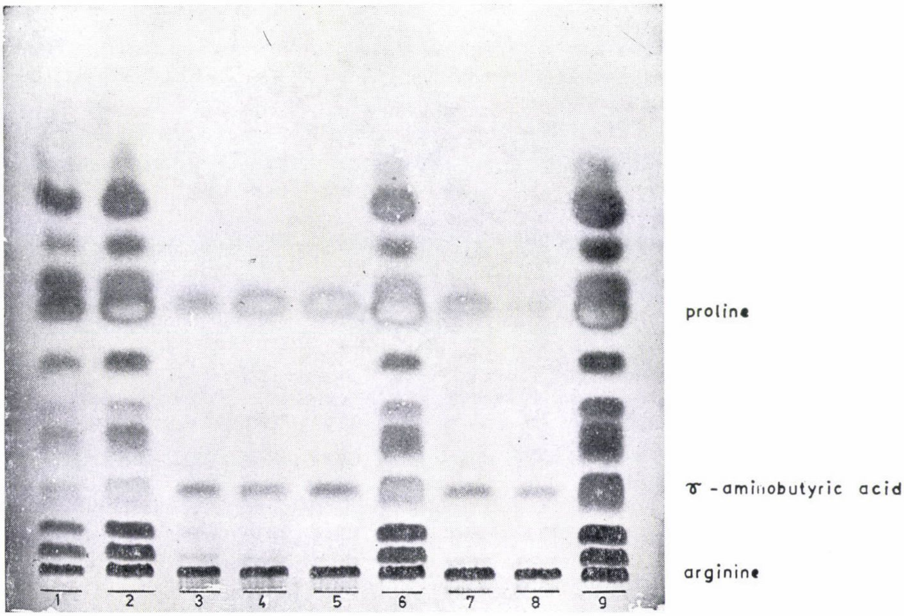

Fig. 3. Separation of free amino acids of grape samples harvested on 19 September 1973 on Fixion 50×8 cation exchange layer. (1), (2), (6), (9): standard amino acid mixtures containing 5, 7, 10, 12 μg per amino acid; (3), (4), (5): Rizlingszilváni control, nitrogen and potassium treatments (40 μl each); Olaszrizling (40 μl); (8): Pirostramini (40 μl). Development with modified collidine ninhydrin (110 °C)

Fig. 6 shows the change of the arginine level in the berries of the different varieties and treatments from 17 July to 15 October as a function of ripening. With the progress of ripening the amount of arginine generally increases in the berries, though the change is not linear; at the beginning of September and October it shows a decreasing tendency.

Similar fluctuation is shown by the curves representing the time-dependent changes of the arginine level in the culture pot treatments of the variety Olaszrizling (Fig. 7).

Table I contains the data of Olaszrizling control, Rizlingszilváni control and nitrogen-treated Rizlingszilváni samples harvested on 18 September as evaluated with the amino acid analyser. The data confirm the results obtained by chromatography, namely that there is no difference in the qualitative amino acid composition of the vine varieties, only the quantities of the individual amino acids varying in them (Table 1).

Comparison of the data unambiguously shows that in ripe fruits supplied with an increased amount of nitrogen proline, arginine and presumably γ-aminobutyric acid accumulate in the largest quantities. Our results essentially agree with those obtained by SHTOEV (1959), LAFON—LAFOURCADE (1962), KLIEWER (1971) and HACHIDZE (1973).

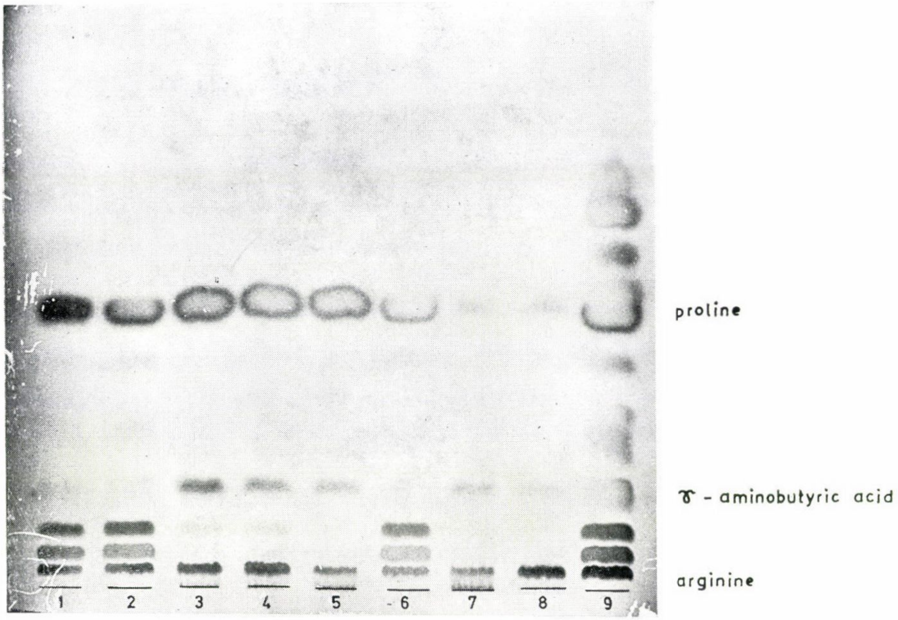

Fig. 4. Separation of free amino acids of grape samples harvested on 2 October 1973 on Fixion 50×8 cation exchange layer. (1), (2), (6), (9): standard amino acid mixtures containing 5, 7, 10, 12 μg per amino acid; (3), (4), (5): Rizlingszilváni control, nitrogen and potassium treatments (40 μl each); (7) Olaszrizling (40 μl); (8): Pirostramini (40 μl). Development with modified collidine ninhydrin (110 °C)

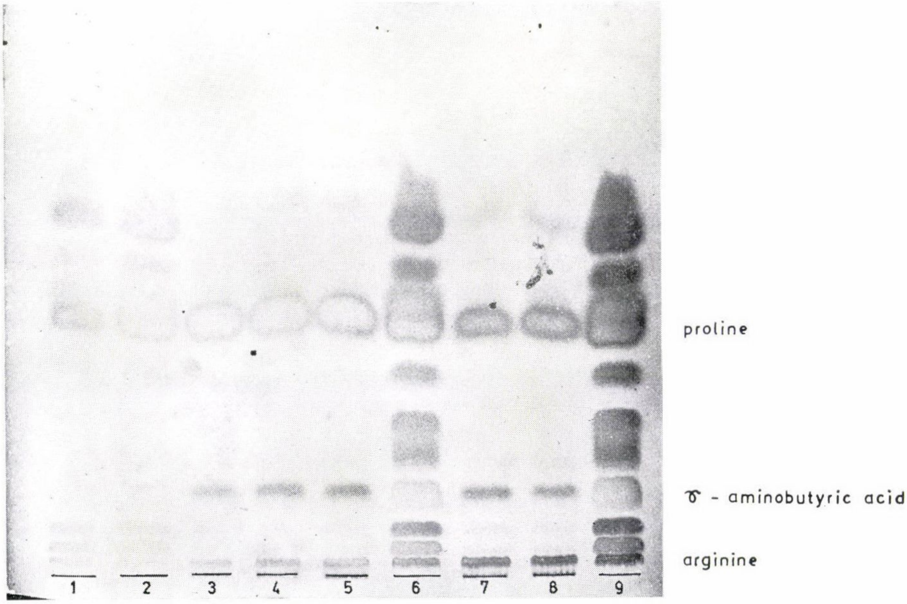

Fig. 5. Separation of free amino acids of grape samples harvested on 15 October 1973 on Fixion 50×8 cation exchange layer. (1), (2), (6), (9): standard amino acid mixtures containing 5, 7, 10, 12 μg per amino acid; (3), (4), (5): Rizlingszilváni control, nitrogen and potassium treatments (40 μl each); (7): Olaszrizling (40 μl); (8): Pirostramini (40 μl). Development with modified collidine ninhydrin (110 °C)

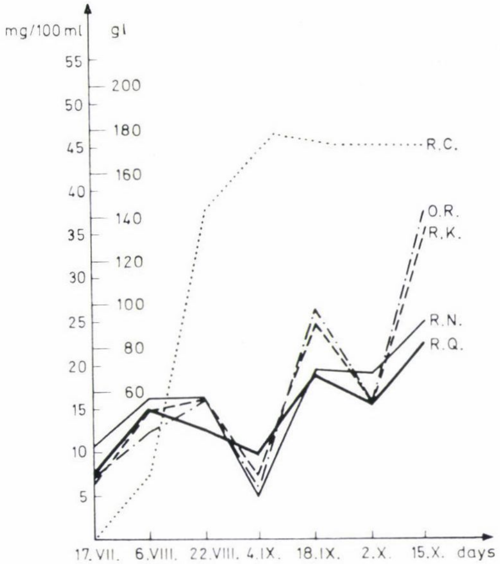

Fig. 6. Changes in the arginine content of grape berry in the course of ripening (mg/100 per variety and treatment). RC: reducing sugar (g/l); OR: Olaszrizling; R0: Rizlingszilváni control; RN: Rizlingszilváni nitrogen treatment; RK: Rizlingszilváni potassium treatment

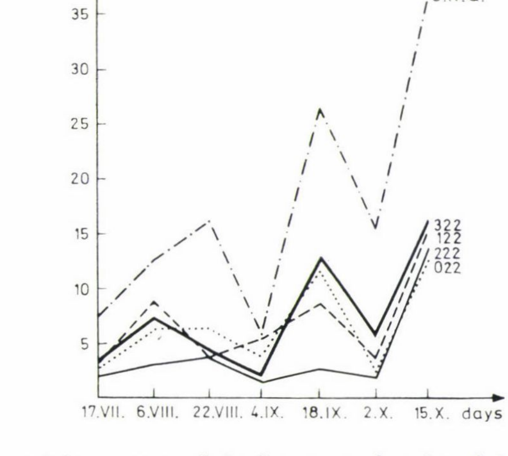

Fig. 7. Changes in the arginine content of vine berry as a function of ripening, in the different Olaszrizling treatments. ORO: outdoors Olaszrizling control; (022), (122), (222), (322): Olaszrizling culture pot treatments. (Increasing rates of nitrogen fertilization)

Table 1

Evaluation by amino acid analyser of Olaszrizling control, Rizlingszilváni control and nitrogen-treated Rizlingszilváni samples harvested on 18 September 1973

Amino acids	Olaszrizling control		Rizlingszilváni control		Rizlingszilváni nitrogen-treated	
	µmole/5 ml	mg/100 ml	µmole/5 ml	mg/100 ml	µmole/5 ml	mg/100 ml
Lys	1.8	5.26	4.7	13.74	5.5	16.08
His	in traces	—	—	—	—	—
NH_3	26.0		24.4		19.6	
Arg	1.9	6.62	2.2	7.66	2.0	6.97
Asp	1.1	2.93	0.7	1.86	1.0	2.66
Thr	4.5	10.71	3.4	8.09	4.7	11.18
Ser	3.0	6.30	3.2	6.72	3.6	7.56
Glu	1.2	3.29	1.1	3.01	1.4	3.84
Pro	4.0	9.21	6.5	14.96	24.0	55.26
Gly	0.4	0.60	0.6	0.90	0.6	0.90
Ala	5.1	9.08	2.2	3.92	2.7	4.81
Cys	+	+	+	+	+	+
Val	0.8	1.87	0.6	1.40	0.7	1.64
Ile	0.4	1.05	0.3	0.78	0.4	1.05
Leu	0.5	1.31	0.6	1.57	0.5	1.31
Tyr	0.2	0.72	0.3	1.08	0.3	1.08

Discussion

Owing to the low number of data and repetitions our pilot character experiments cannot be evaluated mathematically, nor can far-reaching conclusions be drawn from the results. However, our investigations confirm the biochemical and physiological correlations existing between the arginine, proline, γ-aminobutyric acid and glutamic acid, which are of outstanding importance in the development processes of the grape berry (KLIEWER 1969).

The accumulation of free amino acids at the stage of ripening is connected with the biochemical process when the protein synthesis of the nitrogen compounds, which starts with the development of the berry, decreases after ripening. Of the free amino acids arginine and proline are of particular importance in the ripening process of the grape berry. The biosynthetic pathway of the arginine turnover is presumed to start from the glutamic acid (Fig. 8).

The transformation of the arginine mostly takes place in two ways:

1. By decarboxylation it is broken down to putrescine amines, then through γ-aminobutyric acid enters the citrate cycle.

2. Under the influence of arginase it splits to carbamide and ornithine. By transamination from the ornithine glutamic acid-semialdehyde is produced, which by oxidation is transformed into glutamic acid, and with the water removed is cyclized to pyrrolinecarboxylic acid. The latter compound may be reduced to proline.

It seems probable that the arginine transformation takes place through the second pathway in the vine too. This is supported by the results of our investigations, which prove that in the period of ripening glutamic acid and arginine change inversely.

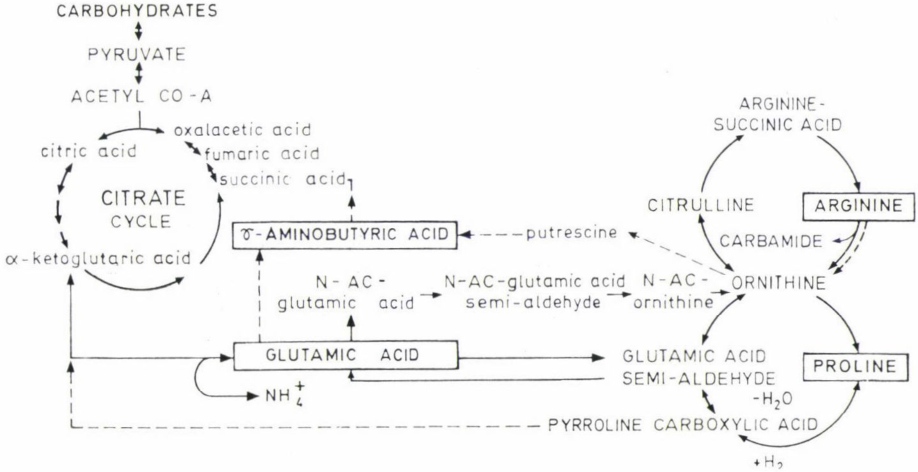

Fig. 8. Interrelations and major biosynthetic pathways in the intermediary metabolism of arginine, proline, γ-aminobutyric acid and glutamic acid (according to NICHOLSON 1969)

The rapid accumulation of proline during the vegetation period can be explained by its being produced from other amino acids through an intermediary material exchange. Arginine reduction and proline increase in overripe fruit indicate that proline may have been synthesized from arginine through the ornithine cycle.

Acknowledgements

We are indebted to Pál Kozma, academician, university professor and head of department, for the great support given to our work; to Ernő Tyihák, research worker of the Research Institute for Medicinal Plants, and to Ernő Dworschák, research worker of the Institute of Nutrition, for their theoretical and practical assistance in the amino acid analyses; and to László Zsoldos, head of the Central Laboratory, for his help in applying the amino acid analyser.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (3—4), pp. 309—318 (1976)

PERFORMANCE OF SOME GENOTYPES OF LUCERNE UNDER WIDE AND NARROW SPACED PLANTING

I. HERITABILITY OF FORAGE YIELD AND RELATED TRAITS AND INTERRELATIONSHIPS AMONG TRAITS

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The objectives of this study were to determine the heritability of the important traits of different types of growth habit of lucerne under wide and narrow spaced planting. The material used were 4 clones representing erect, semi-erect, semi-prostrate, and prostrate types of growth habit. Open-pollinated seed from the 4 clones was sown, and selected plants based upon the highest dry matter yield were propagated vegetatively and transplanted into the experimental field under two spaced planting conditions. Heritability estimates in the narrow-sense for the total of three cuts of different traits were obtained from the analysis of variance. Phenotypic and genotypic correlation coefficients were computed from phenotypic and genotypic covariance and variance. Plant height was of high heritability estimates of 112, 107 and 97 per cent under wide spaces corresponding to 37, 61 and 44% under narrow spaces for C-37, C-636, and C-1474 clones, respectively. Heritabilities estimated for number of stems per plant of C-244, C-636 and C-1474 clones were higher under wider spacing. Heritabilities obtained for dry matter yield and dry stem yield were lower under wider spaces for C-37 and C-244, vice-versa estimates were computed for the other two clones. Heritability estimates for dry leaf weight were nearly the same under the two planting systems, except for the prostrate type (C-1474) which was higher under wider spacing. Most phenotypic correlations among attributes were positive and significant under the two planting systems. Stronger associations between plant height and green yield, dry matter yield and dry stem yield, dry matter yield and dry leaf yield were obtained under close spaces.

Introduction

Selection in forage crops is usually based on the performance of individual plants or clones in spaced nurseries; a system of planting which excludes or depresses intergenotypic competition arises among plants in dense stands of such crops. The value of such selection, thus, depends on the consistency of appraisal of the genetic potential of the material under selection irrespective of the planting system. The present study provides relative information to the effect of spaced planting on the heritability of traits and on phenotypic and genotypic correlations among pairs of traits measured for the same clonal material under two spaced planting systems.

The primary concern of heritability estimation is the assessment of that portion of the variation in a particular trait which is attributed to genetic causes. Lush (1940) has defined broad-, as well as narrow-sense heritabilities. Broad-sense heritability refers to the ratio of total genotypic variability to phenotypic variability. On the other hand, restricted or narrow-sense heritability involves the part of the genotypic variance which pertains to the additive effect of genes. An adequate estimation of narrow-sense heritability is very important to the breeder since the additive genetic effect is normally the only fraction of the genetic variance of value for selection progress (LERNER 1955).

Forage crop breeders have used various methods for heritability estimation. The methods used differ according to the mode of reproduction, mating system, and other biological features of the species investigated. Broad-sense heritability is obtained from the relationship between environmental and genotypic variances estimated for a sample of genotypes or progenies replicated in space and/or time. Narrow-sense heritability can be estimated from: 1. parent-offspring regression (LUSH 1940); 2. polycross progeny variance (THOMAS—KERNKAMP 1954); and 3. Combining ability analysis (KALTON—LEFFEL 1955).

The parent-offspring regression estimates one half the heritability if the parents are non-inbred. If the parents show any degree of inbreeding, SMITH—KINMAN (1965) showed that the true heritability is less than twice the regression coefficient depending on the degree of inbreeding. The variances in 2. and 3. above, are equivalent to the half-sib covariance. This covariance estimates one quarter of the additive variance plus fraction of the epistatic interaction involving additive effects if the parents are not inbred. If the parents coefficient inbreeding is larger than zero, a larger fraction of the additive genetic variance is estimated as well as part of the dominance variance and its interactions (COCKERHAM 1956).

The assessment of variability in many species is based on observations made on individual plants spaced at distances much wider than found at commercial planting rates. However, if different genotypes do not perform similarly relative to each other in spaced and dense seedings as reported for some species (THEURER—ELLING 1964, HINSON—HANSON 1962, and others), the description of variability from spaced nurseries becomes misleading. In soybeans, HINSON—HANSON (1962), found that at relatively wide spacings, the differential response of genotypes to additional space results in an upward bias heritability of seed yield. They questioned the validity of heritability estimates obtained from spaced nurseries for use in selection for performance in solid seedings.

In Ranger alfalfa (*Medicago sativa* L.), KEHR—GARDNER (1960) conducted replicated tests involving solid stands for a number of randomly chosen clones and their polycross progenies. Based on performance over a 2-year period, the heritability of forage yield was 0.28 and 0.25 under irrigation and dry conditions, respectively.

From the analysis of diallel crosses among six previously selected lucerne clones, **Kehr** (1961) estimated heritability for forage yield as 58 per cent. However, he reported that nonadditive variance was more important than additive variance for this trait.

That heritability estimates can vary with the stage of regrowth of lucerne was indicated by **Frakes** *et al.* (1961). From space-planted replicated clones, heritability values for dry matter yield per plant varied from 73 to 85 per cent depending on the period of regrowth up to 38 days. Similarly, heritability for longest stem ranged from 57 to 68 per cent for the same periods. The results reported by **Scossiroli** *et al.* (1963) for strain L 99/100 of lucerne, showed that heritability estimates can also vary between years for the same material, probably, as a result of genotype—environment interaction. Heritability of plant green weight was 6.3 per cent in the second year of growth, while in the first year variance between plants was smaller than that among clones within plants. Heritability for number of stems per plant was 5.7 per cent in the first year but negatively estimated in the second year. Heritability values were 8.7 and 3.8 per cent for plant height, 10.7 and 19.9 per cent for stem weight, and 78.3 and 17.3 per cent for leaf weight in the first and second years, respectively.

The relationship among traits may also alter with increasing plant density as reported by **Rumbauhg** (1963) for the associations among crown width, length of lonegst stem and stem number in lucerne. He concluded that this alteration may reduce the utility of predictive equations derived from space-planted nurseries.

In other forage legumes the yield of forage exhibits generally lower and more variable heritability than other traits (**Johnson—El-Banna** 1957 and **Radwan** 1970).

Johnson—El-Banna (1957), found that the heritability of plant weight in sweet clover estimated from the regression of S progeny means on parental plants increased gradually from 0.02 to 0.49 through 3 cycles of recurrent phenotypic selection for plant vigour. They explained this rise in heritability on the basis of apparent progressive increase in gene frequency for the character selected.

Radwan (1970), reported that broad-sense heritability of differences among commercial seed lots was 0.46 and 0.88 for forage yield and plant height of the single cut from the Fahl variety of berseem.

Material and Method

This study was carried out in the breeding nursery of the Agricultural Research Institute at Kompolt. The materials used were open-pollinated seeds and stem-cuttings from 4 lucerne clones of diverse genetic origin, represented by erect habit (C-37), semi-erect (C-244), semi-prostrate (C-636), and prostrate habit (C-1474), respectively.

The maternal clones were propagated in a greenhouse in the winter of 1970—71, clonal propagantion was continued until there were approximately 200 cuttings of each one. They were placed in seed boxes after rooting in water. Also open-pollinated seeds from maternal clones were sown in seed boxes in a greenhouse in April, 1971.

Plants from seedlings and uniform rooted cuttings were cut at 7 cm height and were transplanted in alternative rows to three space-planted field experiments in randomized complete block design in June 1971; with 4 replications for each one.

Both plant-to-plant and row-to-row distances were 30, 20, and 10 cm. Henceforth: we will refer to the three spaces as wide, medium, and narrow for 30, 20, and 10 cm, respectively. Each half-sib plant and maternal parent-cutting was represented by one 10-plant row in each of the replication under the three spaced-planting experiments.

The presence of one-genotype rows on the two sides of the seedling-row gave a chance to half-sib plants within a row to be under equal conditions of competition.

One border-row was transplanted on each side of the experiment and three cuts consisting of two in 1971 and one in 1972 were taken. The forage yield of singe plants and other related traits was based on harvests at about the 1/10 bloom stage.

On the basis of the total dry yield of three cuts per plant, the best half-sib plant within a row, under the three space-planted experiments, was selected and transferred after seed harvesting to the greenhouse in the winter of 1972—1973.

Hundred stem-cuttings were taken from each selected plant and maternal clone, too. The uniform 30 cuttings in their root vigour were transplanted to the experimental field in May, 1973. A total of 8 experiments in randomized block designs with three replications were established.

Each replication consisted of a 5-plant row of parental clones and 11 or 12 selections. This gave a total of 13 experimental rows within replication from semi-erect and semi-prostrate types, and 12 from erect and prostrate types, as one selection had died. Selections from each type were compared in separate experiments under only wide and narrow spaces with the same randomization under the two conditions within replication with guard row on each side of the experiment.

Three cuts were taken until September, 1973; and the unit of observation was a 5-plant row. Stand counts per row were made after each cut; and yield was expressed as grams of green and dry matter yield, dry stem, and dry leaf weight per plant.

Samples of 100 or less grams were used for leaf/stem separation and dry weight calculations. The leaf/stem separation was made by separating the leaflets. Leaf stems were calculated with the main stem as stem weight. Stems and leaves were dried until the stable weight and the total of the two components gave the dry matter weight.

Other characteristics measured were plant height and number of stems. Plant height in centimeters was obtained by measuring the longest stem from the soil surface to the highest tip for single plants within a row, but average plant height was used in computing. The number of stems arising from the basal nodes was calculated after cutting and was expressed as the number of stems per plant.

Heritability was estimated for the total of the three cuts of different traits under wide and narrow spacing from components of variance derived from analysis of variance in the formula given by KEHR—GARDNER (1960). Heritability was computed as:

$$H = 4\sigma^2 p/\sigma^2 p + \sigma^2 b + \sigma^2 e, \quad \text{where}$$

$\sigma^2 p$, $\sigma^2 b$ and $\sigma^2 e$ are the components of variance for progenies, replications, and errors, respectively.

The variance for progenies was multiplied by 4 to obtain additive variance, which is based on the assumption that progeny variance can be estimated to be 1/4 of the additive genetic variance.

Genotypic and phenotypic correlation coefficients among traits for the total of three cuts were calculated from variance-covariance analysis for pairs of traits of the progenies grown in a wide and narrow space-planted area. The phenotypic correlation was computed by dividing the mean products for progenies of each pair of characters by the geometric mean of the mean squares for both traits. The genotypic correlation coefficient was similarly computed from the corresponding genotypic components of covariance and variance, respectively.

Results

Comparing estimates obtained for each type of growth habit in wide and narrow spaced-planting experiments, heritability was found to clearly differ under the two plantings (Table 1). Values exceeding unity were computed for the plant height of the erect type (1.120) and the semi-prostrate type (1.076). Heritabilities for the number of stems under wide spaces for semi-erect and semi-prostrate types were about twice those estimated under narrow spaces. Values estimated under wide spaces were .840 and .684 for semi-erect and semi-prostrate types, respectively; and .428 and .380 under narrow spaces for the two types in the same order as above. For erect and prostrate types similar estimates were computed under the two plantings. Dry stem weight was a highly heritable trait under narrow spaces compared with that under wide spaces for erect, semi-erect, and semi-prostrate types, respectively. Values computed were .400, .496, and .728 under narrow spaces, and .212, .128, and .688 under wide spaces for the three types in the same order as above. For the prostrate type, heritability estimated under wide spaces, (.840) was twice that estimated under narrow spaces (.420) for dry stem weight, values for the number of stems were similar in the two experiments. Heritabilities of green yield were in the same line with those for dry matter yield, and were

Table 1

Heritability estimates from wide and narrow spaced-plantings for the traits of 4 types of growth habit of lucerne

Clone	Spaces	Traits					
		Plant height	Green yield	Number of stems	Dry weight	Dry stem weight	Dry leaf weight
C-37	W	1.120	.284	.312	.356	.212	.524
(erect)	N	.372	.464	.356	.368	.400	.404
C-244	W	.568	.272	.840	.240	.128	.460
(semi-erect)	N	.808	.512	.428	.516	.496	.520
C-636	W	1.076	.600	.684	.608	.688	.520
(semi-prostrate)	N	.612	.576	.380	.624	.728	.464
C-1474	W	.976	.816	.560	.840	.840	.880
(prostrate)	N	.448	.440	.552	.512	.420	.564

W = wide
N = narrow

higher under narrow than under wide spaces for erect and semi-erect types, while they were in the opposite line in the two experiments for prostrate and semi-prostrate types. Leaf weight as a character was highly heritable under wider spaces for erect, semi-prostrate, and prostrate types, and larger values were computed under narrow than under wide spaces for the semi-erect type.

Phenotypic and genotypic correlations among all pairs of 6 traits of interest to the breeder are presented in Tables 2/a—d. In most cases phenotypic correlations were smaller under narrow than under wide spaces for erect and semi-erect types, while they were larger under narrow than under wide spaces for prostrate and semi-prostrate types. Insignificant associations for plant height with the number of stems were obtained under wide and narrow spaces for the semi-erect type but only under narrow spaces for the erect type.

Table 2/a

Phenotypic (right) and genotypic (left) correlations among six attributes in the progenies of C—37 clone under two spaced-plantings

		Plant height	Green yield	Number of stems	Dry matter weight	Dry stem weight	Dry leaf weight
Plant height	W	—	.815**	.665*	.785**	.820**	.690*
	N	—	.448	.383	.622*	.741**	.716**
Green yield	W	.069	—	.922**	.992**	.922**	.936**
	N	.988	—	.784**	.985**	.968**	.967**
Number of stems	W	—.004	.501	—	.940**	.921**	.911**
	N	.300	.703	—	.699*	.964*	.796**
Dry matter weight	W	.072	.990	.578	—	.934**	.964**
	N	.997	.998	.879	—	.905**	.896**
Dry stem weight	W	.197	.979	.668	.996	—	.902**
	N	.948	.993	.738	.996	—	.858**
Dry leaf weight	W	—.036	.995	.520	.994	.997	—
	N	.782	.972	.755	.998	.965	—

W = wide space
N = narrow space
*, ** = Significant from zero at the 5% and 1% levels of probability, respectively

The genotypic associations between plant height and other traits under wide spaces are negative or rather weak, whereas fair to strong positive correlations were found for the same relationships under narrow spaces.

Table 2/b

Phenotypic (right) and genotypic (left) correlations among six attributes in the progenies of C—244 clone under two spaced plantings

		Plant height	Green yield	Number of stem	Dry matter weight	Dry stem weight	Dry leaf weight
Plant height	W	—	.753**	.573*	.731**	.737**	.722**
	N	—	.800**	.573*	.807**	.775**	.809**
Green yield	W	—.332	—	.822**	.990**	.979***	.955**
	N	.899	—	.761**	.708**	.955**	.995**
Number of stems	W	—.826	.691	—	.841**	.789**	.869**
	N	—.160	.222	—	.759**	.729**	.765**
Dry matter weight	W	—.534	.962	.867	—	.985**	.970**
	N	.893	.991	.238	—	.588*	.995**
Dry stem weight	W	—.250	.939	.701	.949	—	.915**
	N	.964	.998	.150	.998	—	.955**
Dry leaf weight	W	—.352	.912	.936	.969	.848	—
	N	.745	.980	.384	.985	.983	—

Table 2/c

Phenotypic (right) and genotypic (left) correlations among six attributes in the progenies of C—636 clone under two spaced plantings

		Plant height	Green yield	Number of stems	Dry matter weight	Dry stem weight	Dry leaf weight
Plant height	W	—	.835**	.853*	.886**	.861**	.791**
	N	—	.869**	.952**	.871**	.861**	.799**
Green yield	W	.799	—	.852**	.990**	.978**	.971***
	N	.817	—	.769**	.992**	.974**	.753**
Number of stems	W	.519	.993	—	.956**	.839**	.870**
	N	.584	.759	—	.770**	.731**	.796**
Dry matter weight	W	.789	.995	.769	—	.989**	.975**
	N	.832	.998	.772	—	.989**	.983**
Dry stem weight	W	.860	.955	.726	.979	—	.934**
	N	.872	.997	.761	.994	—	.955*
Dry leaf weight	W	.646	.971	.828	.941	.856	—
	N	.819	.293	.778	.969	.965	—

Table 2/d

Phenotypic (right) and genotypic (left) correlations among six attributes in the progenies of C—1474 clone under two spaced-plantings

		Plant height	Green yield	Number of stems	Dry matter weight	Dry stem weight	Dry leaf weight
Plant height	W	—	.646*	.494	.708**	.706**	.681**
	N	—	.683*	.647*	.770**	.785**	.737**
Green yield	W	.539	—	.847**	.978**	.986**	.964**
	N	.660	—	.804**	.929**	.977**	.960**
Number of stems	W	.135	.646	—	.821**	.781**	.817**
	N	.195	.778	—	.799**	.806**	.749**
Dry matter weight	W	.516	.998	.753	—	.977**	.959**
	N	.211	.997	.802	—	.919**	.982**
Dry stem weight	W	.694	.929	.657	.972	—	.877**
	N	.742	.994	.763	.988	—	.956**
Dry leaf weight	W	.200	.907	.801	.938	.807	—
	N	.542	.951	.826	.980	.930	—

Near to unity phenotypic and genotypic associations were found in all types between leaf and stem weight and dry matter yield under the two spaced plantings, and also between green and dry matter yield.

Discussion

For the rest of the characters studied there was a narrow-sense estimate exceeding the unity. Such estimates are considered incorrect by definition since narrow-sense heritability estimates additive genetic variance which is a part of the total genetic variance. Estimates of heritability are expected to be biased upward if the parents of the progenies used are partially inbred (Potts—Holt 1967, Smith—Kinman 1965). The extent of bias in the estimates reported here cannot be determined since it is not known to what degree the parents used are inbred. However, it is expected that these estimates are somewhat larger than supposed to be since self-fertility seems to be common in lucerne (Brink—Cooper 1938, Cooper—Brink 1940, Wilsie 1951, Wilsie—Skory 1948). Therefore, they should represent the upper limit for heritabilities in the materials studied. The second reason is that the progenies tested here are half-sibs, consequently: have only half of their genes in common. Therefore the within progeny variance (σ^2e) used as a measure of environmental variance is in fact inflated with some genotpyic variance.

Stem number and stem weight were not inherited in the same line, and stem weight was a more heritable character under narrow than under wide spaces for erect, semi-erect and semi-prostrate types, while the number of stems was a more heritable trait under wide than under narrow spaces. These results lead us to suggest that the factors controlling stem number per plant were more affected by space area than other traits, and selection for this trait would be better applied under spaces similar to those of commercial production than under wider spaces in the nursery; and selection for stem weight may be more effective in improving yield than selection for number of stems.

The strength of the association among certain traits was shown to be influenced by the planting system, which is in line with the findings of RUMBAUGH (1963) for lucerne. Thus, selection for performance in solid seedings, based on selection indices constructed from relationships in spaced nurseries, may not be rewarding for two reasons. First, a strong relationship in spaced nurseries may be of low prediction in dense planting. Second, there are different responses of genotypes to spacing. The negative values listed in Tables 2/a and 2/b could be taken as an indication of the fact that genotypes may respond differently to the system of planting. However, because most of the traits show a stronger association with yield under narrow than under wide spaced planting, more genotypes should be expected in improving yield from selection for these traits under narrow spaced planting.

Based upon the correlations found here, a scheme of serial elimination of undesirable genotypes is suggested for the selection of parental clones in order to minimize the labor of evaluating large populations. These correlations indicate that improved forage yield in these materials will not be accompanied with a reduction of the leaf/stem ratio and selection for only leaf yield will not be effective in improving this important attribute.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (3—4), pp. 319—333 (1976)

SURVIVAL, DEVELOPMENT AND PLANT DAMAGE OF THE EUROPEAN CORN BORER ON OPAQUE-2 AND NORMAL MAIZE

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Survival, development and plant damage of the European corn borer, *Ostrinia nubilalis* (Hbn.), were compared in opaque-2 corn varieties and their normal counterparts, using artificial infestation with egg masses. Seven commercial and 2 open-pedigree opaque-2 hybrids as a group had 17 to 26% more 1st-brood borers and 13% more tunnels than the normal hybrid group. Four open-pedigree opaque-2 hybrids as a group had 17% more 1st-brood borers and 16% more tunnels than the normal group, and borer development was 0.11 to 0.28 stadia greater on opaque-2 hybrids. The 5 opaque-2 inbred parents of the hybrids have 9% more 1st-brood borers and 21% more tunnels than normal inbreds, and borer development was 0.10 stadia greater on opaque-2 inbreds. The degree of greater susceptibility of opaque-2 varieties to the 1st-brood was found to vary with the pedigree. Hybrids with B14A as one of the parents showed the "reverse response" with 6 to 15% fewer borers on the opaque-2 hybrid group. However, borer development was 0.07 to 0.23 stadia greater on the opaque-2 B14A hybrids. Four open-pedigree opaque-2 hybrids as a group had 8 to 10% more 2nd-brood borers and 16% more tunnels than the normal hybrid group. Opaque-2 and normal inbred groups gave similar results with 2nd-brood infestation.

Introduction

Changes in agronomic characters in corn (*Zea mays* L.) or the introduction of genes for corn improvement may alter the level of resistance to the European corn borer, *Ostrinia nubilalis* (Hübner). The discovery of the effects of the opaque-2 gene on protein quality in corn (Mertz *et al.* 1964) has led to the development and release of opaque-2 corn hybrids (high-lysine hybrids). Such hybrids may become widely used in the future. The opaque-2 gene increases the concentration of the nutritionally important amino acids lysine and tryptophan in corn endosperm.

The effects of the open opaque-2 gene on 1st-brood European corn borers were determined in an earlier study (Windels *et al.* 1972) which included 6 commercial opaque-2 hybrids and their normal counterparts. Borer survival was significantly higher on the opaque-2 hybrid group with 25 and 33% more borers at 2 and 4 weeks after infestation, respectively. The opaque-2 hybrid group also had significantly greater plant damage with 54, 22 and 18% more tunnels than the normal group at 2, 4 and 10 weeks after infestation, respectively. Of particular significance was one hybrid pair showing the "reverse response" of lower borer survival and fewer tunnels on the opaque-2 hybrid; hence the genetic background may modify the effects of the opaque-2 gene.

Since 1st-brood borers feed largely on leaf tissue, analyses for lysine were made for leaf tissue from opaque-2 and normal hybrids (Windels *et al.* 1972). No significant differences were found. Nelson (1969) reported that there was little or no difference in the amino acid composition of the proteins from leaf tissue or pollen for opaque-2 and normal genotypes.

The present study was conducted to further investigate the effects of the opaque-2 gene on 1st-brood borer survival, development and damage to corn plants and to try to identify genetic material responsible for the "reverse response". Genetic material showing the "reverse response" may be useful in overcoming the greater susceptibility of opaque-2 hybrids. The effects of the opaque-2 gene on the 2nd-brood were also investigated. The relative resistance to both the 1st and 2nd-broods based on several criteria was determined for hybrids and inbreds.

Materials and methods

A series of experiments was conducted at the University of Minnesota Southern Experiment Station, Waseca, Minnesota, in 1971, 1972 and 1973. Effects of the opaque-2 gene on European corn borer survival, development and tunnelling were determined through comparisons of opaque-2 varieties and their normal counterparts after manual infestation with egg masses. Randomized complete block designs were used with the number of replications varying with individual experiments. Separate plots were planted for 1st- and 2nd-brood evaluations and for hybrids and inbreds. Entries were planted with a cone planter in single row plots 76 cm wide and plants were thinned to 1 plant/30 cm of row. In each row the first 20 plants showing no sign of natural infestation were used as treatment plants and were manually infested.

Number of borers and tunnels/plant, borer development, and leaf injury ratings were recorded in the experiments; however, not all of these criteria were used in each experiment. Number of borers and tunnels/plant were determined through plant dissections at 1, 2, 3 and 4 weeks after manual infestation. The first 10 manually infested plants were dissected at the first, and the second 10 plants at the second sampling period. All plants were thoroughly examined and the number and estimated instars of living larvae were recorded. In calculating the mean instar, 5th instar larvae that had completed feeding were assigned a value of 6.

Tunnel length was estimated to the nearest 1/4 inch (0.64 cm) and each accumulated inch (2.54 cm) was recorded as one tunnel. Leaf injury ratings were made on a row basis 3 weeks after egg hatch by using the 1—9 rating scale developed by Guthrie *et al.* (1960). Leaf injury ratings were made on those infested plants that were to be dissected at 4 weeks and on special plantings infested for taking leaf injury ratings only. Analyses of variance were computed for number of borers and tunnels/plant, mean borer instar and leaf injury ratings; variety pairs were compared by using the Least Significant Difference test.

Egg mass production and manual infestation methods

Corn borer egg masses were obtained by using the following procedure. Naturally infested corn stalks were kept in cages at St. Paul, Minnesota, through winter and spring months to obtain moths in the summer. Emerging moths were collected from these cages and placed in oviposition cages for egg mass production. For maximum egg production, cages were kept at 28 °C during the day and 23 °C at night with an 18-hour photoperiod; relative humidity was maintained above 90% and drinking water was supplied daily. Months oviposited on sheets of wax paper, and discs containing egg masses were punched out with a mechanical punch.

Early emerging moths were used to obtain egg masses for 1st-brood infestation. Plants were manually infested for 1st-brood evaluation by placing 3 egg masses (approximately 60 eggs) in the black-head stage of development in each plant whorl when plants were approximately 60 to 90 cm tall.

Moth emergence in cages at St. Paul is approximately 2 weeks later than the natural moth emergence at Waseca and as a result manual 1st-brood infestation was 2 weeks behind natural infestation. Due to the large number of egg masses needed, only 1 or 2 replications were infested per day. This procedure also helped to spread out the later work of dissecting the plant.

Moths emerging late from field emergence cages were placed in oviposition cages and kept at 15 °C for up to 2 weeks to delay oviposition. Egg masses producted by these moths were kept at 15 °C for up to 10 days to delay hatching and were used in August for 2nd-brood infestation.

Second-brood infestation differs from 1st-brood infestation by the plant development stage at infestation and by placement of egg masses. To simulate natural 2nd-brood infestation (WINDELS—CHIANG 1975), plants just past the pollen shedding stage were infested with 3 egg masses/plant on the ear leaf and on leaves above and below the ear. Infested plants were sib-pollinated, so there was no change in the genotype of the kernels.

Experiment A: commercial and open-pedigree hybrids

In 1971, 3 opaque-2 and normal hybrid pairs in addition to the 6 pairs evaluated earlier (WINDELS *et al.* 1972) were examined in 3 replications to confirm the general trend of higher borer survival and tunnelling on opaque-2 hybrids. Seven of the pairs were closed pedigree hybrids obtained from commercial seed companies and represented opaque-2 material currently available. Two pairs consisted of open-pedigree hybrids. Only a 1st-brood evaluation, at 2 1/2 and 4 weeks after infestation, was made.

Experiment B: open-pedigree hybrids and inbred lines

The use of closed-pedigree hybrids in Experiment A limited the conclusions that could be drawn concerning their genetic make-up. In Experiment B, open-pedigree hybrids and their inbred parents were examined in 1972 and 1973 for 1st-brood leaf feeding and 1st- and 2nd-brood leaf feeding and 1st- and 2nd-brood borer survival, development and tunnelling. Four open-pedigree opaque-2 hybrids and their normal counterparts and 5 opaque-2 inbreds and corresponding normal inbreds were obtained from Clyde Black and Son, Inc., Ames, Iowa. Hybrids and inbreds were planted in separate blocks with separate plots for 1st- and 2nd-brood evaluations. There were 4 replications each year. Before manual infestation all plots were evaluated for 1st-brood natural infestation, after which all naturally infested plants were removed when rows were thinned to the desired stand. A total of 20 replications was examined for % natural infestation based on plants/row showing leaf feeding injury.

It was not the objective of Experiment B to further establish the greater susceptibility of opaque-2 hybrids as a group, based on only 4 pairs of hybrids. If one or more of the pairs showed the "reverse response" it would be unlikely that the opaque-2 hybrid group would show greater susceptibility than the normal group. The main objective has to identify genetic material showing differences between the opaque-2 and normal genotypes.

Experiment C: B14A in hybrid combination

Results from Experiment B in 1972 suggested that inbred B14A may contribute to the "reverse response" when used in hybrid combination. Three opaque-2 hybrids and their normal counterparts, with B14A as one of the parents, were obtained from Clyde Black and Son, Inc. In 1973, entries were evaluated for response to the 1st brood; there were 4 replications. Borer survival was evaluated at 1, 2 and 3 weeks after manual infestation.

Results

Experiment A: commercial and open-pedigree hybrids with first-brood infestation

The 9 opaque-2 hybrids as a group had 26 and 17% more borers than the normal group at 2 $^1/_2$ and 4 weeks, respectively ($P < 0.05$; Table 1). The opaque-2 versus normal comparison was not significant for number of tunnels at 2 $^1/_2$ or 4 weeks. Number of tunnels at 21/2 weeks was low since borers were only beginning to invade stalk tissue at that time. Number of tunnels at 4 weeks was 13% higher on the opaque-2 group. Four of the 9 hybrid pairs showed significant differences between genotypes within pairs, with more borers or tunnels on the opaque-2 hybrids.

The "reverse response" of a significantly lower number of borers and tunnels on the opaque-2 genotype did not occur; although there was a tendency

Table 1

Number of borers and tunnels per plant at 2 1/2 and 4 weeks after artificial 1st-brood infestation of opaque-2 and normal hybrids in Experiment A (1971)

Hybrid	genotype	2 $^1/_2$ weeks		4 weeks	
		borers	tunnels	borers	tunnels
TX102	0—2	5.4+	1.6	3.5*	4.1**
	N	3.7	1.8	2.2	2.1
TXS102	0—2	3.1	1.1	3.2	3.0
	N	3.2	1.4	3.6	4.1
TXS106	0—2	5.3	3.3	4.4	4.6
	N	4.5	2.4	3.7	4.5
TXS107	0—2	5.4+	2.0	4.5*	4.7**
	N	3.6	1.4	3.1	2.6
TXS114	0—2	2.7	1.4	3.6+	2.8
	N	2.9	1.4	2.5	3.0
PX50	0—2	3.8	2.4	2.4	2.5
	N	3.7	1.9	3.0	3.1
PX480	0—2	4.5	1.9	3.6	4.1
	N	4.3	2.8	3.6	3.9
A619 × A632	0—2	5.3*	2.0	3.7	3.7
	N	2.8	1.6	3.2	3.0
B37 × B14A	0—2	4.2	1.1	2.5	2.5
	N	3.2	0.8	1.9	2.1
Group mean	0—2	4.4*	1.9	3.5*	3.6
	N	3.5	1.9	3.0	3.2

** 1% level of significance
* 5% level of significance
+ 10% level of significance

for fewer borers on opaque-2 hybrids in some cases. Hybrid pair PX480, which gave the "reverse response" in earlier investigations (WINDELS *et al.* 1972), did not confirm these observations at either sampling period.

The relative resistance of hybrids examined can be determined from borer survival and tunnelling. Both genotypes of hybrid pair B37 × Bl4A were the most resistant of the hybrids as based on these criteria. The opaque-2 genotype of TXS107 and both genotypes of TXS106 were the most susceptible.

These results confirm earlier findings that there is greater borer survival and tunnelling on the commercially available opaque-2 hybrids as compared to their normal counterparts. Thus, more extensive damage can be expected in opaque-2 hybrid plantings when natural infestation occurs, and more larvae may survive to contribute to higher populations in future generations. Though none of the hybrids gave a significant "reverse response", results indicate that certain opaque-X2 hybrids such as B37 × Bl4A can be selected that have lower borer survival and plant damage than near isogenic but normal hybrid counterparts.

Experiment B: open-pedigree hybrids and inbred lines with first and second-brood infestation

Hybrids with 1st-brood infestation

Opaque-2 versus normal genotype comparisons were made between hybrid groups and within hybrid pairs. At 2 weeks there were 7% more borers on the opaque-2 hybrid group but this difference was not significant (Table 2). Within only one of the hybrid pairs, W64A × A619, were genotypes different, with more borers on the opaque-2 hybrid ($P < 0.10$).

Mean borer instar at 2 weeks was higher on the opaque-2 hybrid group with borers averaging 0.11 of a stadium older ($P < 0.05$). Although none of the genotype comparisons within pairs were significantly different for mean instar, all 4 hybrid pairs did tend to show advanced borer development on the opaque-2 hybrids.

Number of borers at 4 weeks was low and there were no differences between genotype groups or between hybrid pairs. Number of tunnels at 4 weeks was also low and there were no significant differences between genotype groups, although there were 16% more tunnels on the opaque-2 hybrid group.

At 4 weeks, mean borer instar was higher on the opaque-2 group with borers averaging 0.28 of a stadium older ($P < 0.01$). Borers on all 4 hybrid pairs tended to show greater development on the opaque-2 hybrids.

There were no significant differences for leaf injury ratings between genotype groups or between genotypes of hybrid pairs. Natural infestation

Table 2

Number of borers and tunnels per plant and mean borer instar at 2 and 4 weeks, and leaf injury ratings for hybrids with 1st-brood infestation (1972 and 1973 combined)

Hybrids	genotype	2 weeks		4 weeks			leaf injury rating
		borers	instar	borers	tunnels	instar	
A619 × A632	0—2	5.6	2.76	1.5	2.8	4.89	3.1
	N	4.7	2.71	1.5	2.6	4.65	3.0
B37 × B14A	0—2	5.4	2.80	0.7	1.7	4.95+	5.0
	N	5.9	2.65	0.7	2.1	4.56	5.1
W64A × A619	0—2	8.0+	2.78	0.9	3.3	4.99	3.4
	N	6.1	2.63	1.1	3.1	4.89	3.3
W64A × A632	0—2	9.0	2.73	1.1	3.7*	4.88+	5.1
	N	9.5	2.66	1.0	2.2	4.49	4.9
Group mean	0—2	6.98	2.77*	1.03	2.85	4.93**	4.2
	N	6.53	2.66	1.03	2.46	4.65	4.1

** 1% level of significance
* 5% level of significance
+ 10% level of significance

of the hybrids was quite variable, ranging from 6 to 14% with means of 9.5 and 9.3% for opaque-2 and normal genotypes, respectively. There was no apparent preference for either genotype by moths of the spring moth flight.

The 4 hybrid pairs differed for borers' plant at 2 and 4 weeks, tunnels/plant at 4 weeks, and leaf injury ratings. These measurements indicate the relative resistance of the hybrid pairs and tissues examined. Different tissues are involved at different sampling periods. Borer survival at 2 weeks superimposes subsequent feeding on sheath and stalk tissue. Hybrid pairs W64A × A632 and W64A × A619 had the highest borer survival at 2 weeks and the highest number of tunnels at 4 weeks. Pair W64A × A632 also had a relatively high leaf injury rating. Hybrid pair B37 × B14A had low borer survival and tunnelling, although leaf injury ratings were high. Hybrid pair A619 × A632 had a low number of borers at 2 weeks and a low leaf injury rating, however, borer survival and tunnelling at 4 weeks were high. Differences in resistance were greater among than within pairs; it follows that opaque-2 hybrids, such as B37 × B14A, could be selected that have lower borer survival and plant damage than normal hybrids of a different genetic background.

The results of Experiment B show that opaque-2 hybrids are slightly more susceptible than normal hybrids to the 1st brood. Survival development and tunnelling of borers were greater on the opaque-2 hybrids. In none of the hybrid pairs was either genotype consistently more susceptible than its counterpart for the different criteria. There was no significant "reverse response" shown by any of the hybrid pairs.

Inbreds with 1st-brood infestation

At 2 weeks there was no significant difference in the number of borers for the opaque-2 and normal inbred groups (Table 3). There were differences between the genotypes of A619 and more borers on the normal genotype of A619 and more borers on the normal genotype of B14A ($P < 0.01$). There were no significant differences in mean instar at 2 weeks for the genotype of inbred groups or genotype within inbred pairs.

Table 3

Number of borers and tunnels per plant and mean instar at 2 and 4 weeks, and leaf injury ratings for inbreds with 1st-brood infestation (1972 and 1973 combined)

Inbreds	genotype	2 weeks		4 weeks			leaf injury rating
		borers	instar	borers	tunnels	instar	
A619	0—2	6.0**	2.70	1.4	4.5	5.13	2.9
	N	3.7	2.69	1.3	3.6	4.90	2.5
A632	0—2	8.1	2.86	2.2*	4.8	5.14	5.1
	N	7.7	2.89	1.6	4.0	5.10	5.5
B14A	0—2	6.2**	2.76	1.2	3.2	5.16	6.5**
	N	8.7	2.63	1.4	3.1	4.91	5.5
B37	0—2	3.3	2.75	0.6	1.7	4.81	6.5
	N	3.5	2.73	0.5	1.4	4.75	7.0
W64A	0—2	5.9	2.80	1.2	4.7*	5.25	6.0+
	N	6.1	2.92	1.4	3.5	5.31	6.6
Group mean	0—2	5.91	2.78	1.32	3.76*	5.10	5.40
	N	5.94	2.77	1.21	3.10	5.00	5.42

** 1% level of significance
* 5% level of significance
\+ 10% level of significance

At 4 weeks the number of borers/plant was low. The opaque-2 inbred group had 9% more borers than the normal group. The opaque-2 inbred group had 21% more tunnels/plant at 4 weeks than the normal group ($P < 0.05$). All 5 inbred pairs had slightly more tunnels on the opaque-2 inbreds.

There were no significant differences for genotype between inbred groups or within inbred pairs for mean borer instar at 4 weeks. Four of the 5 inbred pairs had slightly higher mean instar on the opaque-2 inbreds.

There were no differences in leaf injury ratings for the genotype groups, however, there were differences for genotypes within inbred pairs. Inbred pair W64A had a lower leaf injury rating on the opaque-2 genotype ($P < 0.01$) and B14A had a higher rating on the opaque-2 genotype ($P < 0.01$). These differences for genotype within inbred pairs did not correspond with significant differences based on borers/plant at 2 weeks for B14A and tunnels/plant at 4 weeks for W64A. Leaf injury ratings for normal inbreds compared favourably with those reported by GUTHRIE—DICKE (1972).

Natural 1st-brood infestation of inbreds was lower than that for hybrids, as the less vigorous and shorter inbred plants are less attractive to ovipositing moths. Natural infestation ranged from 2 to 8% with means of 5.0 and 3.6% for opaque-2 and normal genotype groups, respectively.

Significant differences were found among the 5 inbred pairs for borers/plant at 2 and 4 weeks, tunnels/plant at 4 weeks, mean instar at 2 and 4 weeks, and leaf injury ratings. Inbred pair B37 had the lowest borer survival at 2 and 4 weeks, the lowest number of tunnels at 4 weeks, and the lowest mean instar for borers of all the inbred pairs at 4 weeks. Inbred pair A632 had the highest borer survival at 2 and 4 weeks, and the highest mean instar at 2 weeks. Inbred pair A619 had a resistant leaf injury rating and the lowest rating of the inbred pairs.

Comparison of hybrids and inbreds with 1st-brood infestation

Generally, opaque-2 hybrids and inbreds with 1st-brood infestation were more susceptible than were the normal genotypes. In 3 of the 4 hybrid pairs and 3 of the 5 inbred pairs there were significant responses of greater borer survival, development or tunnelling in the opaque-2 hybrids. In none of the hybrids pairs and in only one inbred pair was the opaque-2 genotype significantly less susceptible, based on these criteria.

In hybrid pair A619×A632 evaluated in Experiment A there were significantly more borers at 2 weeks on the opaque-2 hybrid and the number of borers at 4 weeks and tunnels at 2 and 4 weeks also tended to be higher on the opaque-2 hybrid. In Experiment B, A619×A632 also tended to show higher borer survival, borer development and plant damage on the opaque-2 hybrid. Both parent inbred pairs A619 and A632 followed the same trend with opaque-2 genotypes more susceptible than their normal counterparts.

Hybrid pair B37×B14A was also examined in Experiment A, and although the opaque-2 hybrid had slightly higher borer survival and tunnelling, the differences were not significant. In Experiment B there were no significant differences between genotypes of B37×B14A for borer survival and tunnelling, although the number of borers at 2 weeks and tunnels at 4 weeks were slightly lower on the opaque-2 hybrid. Inbred parent B37 showed no significant differences between genotypes, and B14A had significantly fewer borers on the opaque-2 inbred at 2 weeks.

In hybrid pair W64A×A619 there were slight differences between genotypes, with slightly higher borer survival at 2 weeks on the opaque-2 hybrid. This difference was similar to that of the opaque-2 version of inbred A619.

In hybrid pair W64A × A632, the opaque-2 hybrid had a higher number of tunnels than its normal counterpart. In inbred pairs W64A and A632 there were also more tunnels on the opaque-2 genotypes.

The influence of the inbred parents on their respective hybrids is easily discernible. Borer survival for B37 × B14A was low at 2 weeks and the lowest of the hybrid pairs at 4 weeks. Inbred pair B37 had the lowest number of borers and tunnels of all the inbred lines. Although B37 × B14A and inbred B37 had low borer survival and tunnelling, leaf injury ratings were among the highest of the varieties examined. This may be due to resistance to late instar larvae. GUTHRIE *et al.* (1960) reported that B14 (B14A is a B14 backcross for rust resistance) has a susceptible leaf injury rating; however, it apparently has resistance to late instar larvae which results in low numbers of borers reaching maturity.

Hybrids with 2nd-brood infestation

At 2 weeks there were 8% more borers on the opaque-2 hybrid group, however, this difference was not significant (Table 4). Only one hybrid pair, W64A × A632, had differences between genotypes with more borers on the opaque-2 hybrid ($P > 0.01$).

Borers of the 2nd-brood developed faster and entered the stalk earlier. Consequently, there was measurable tunnelling at 2 weeks, although most of the tunnels were merely short probes into the stalk. Number of tunnels at 2

Table 4

Number of borers and tunnels per plant and mean borer instar at 2 and 4 weeks for hybrids with 2nd-brood infestation (1972 and 1973 combined)

Hybrids	genotype	2 weeks			4 weeks		
		borers	tunnels	instar	borers	tunnels	instar
A619 × A632	0—2	14.9	3.0	3.74	6.7	5.9+	5.04
	N	14.0	3.3	3.80	6.6	7.2	5.00
B37 × B14A	0—2	8.5	2.6	3.67	6.4	3.8	5.13
	N	10.4	2.0	3.65	6.4	4.5	4.91
W64A × A619	0—2	12.1	3.7	3.69	6.9+	7.2	4.96
	N	11.4	3.8	3.71	5.7	6.4	4.98
W64A × A632	0—2	14.4**	4.0**	3.81**	7.1+	5.4	4.95
	N	10.3	2.4	3.61	6.0	4.9	4.89
Group mean	0—2	12.45	3.33+	3.73	6.77+	5.55	5.02
	N	11.52	2.88	3.69	6.13	5.75	4.94

** 1% level of significance
* 5% level of significance
+ 10% level of significance

weeks was significantly higher on the opaque-2 group of hybrids with 16% more tunnels ($P < 0.10$). Again, in hybrid pair W64A × A632, the opaque-2 genotype showed greater susceptibility with more tunnels than the normal hybrid ($P < 0.01$).

There was no significant difference for mean instar at 2 weeks for the genotype groups. Hybrid pair W64A × A632 again had significant differences between genotypes with a large mean instar on the opaque-2 hybrid ($P < 0.01$)

Number of borers at 4 weeks was higher on the opaque-2 group, with 10% more borers ($P < 0.10$). Two hybrid pairs, W64A × A632 and W64A × A619, had higher numbers of borers on the opaque-2 genotypes ($P < 0.01$).

Number of tunnels at 4 weeks was slightly lower on the opaque-2 group but this difference was not significant. There were fewer tunnels on the opaque-2 genotype of hybrid pair A619 × A632 ($P < 0.10$).

Mean instar was not significantly different for the genotype groups at 4 weeks but seemed slightly higher on the opaque-2 group. There were no significant differences between genotypes within hybrid pairs for mean instar.

Significant differences were found among the 4 hybrid pairs for borers (plant at 2 weeks, tunnel) plant at 2 and 4 weeks, and mean borer instar at 2 weeks. Hybrid pair B37 × B14A was primarily responsible for these differences with lower borer survival, development and tunnelling than the other hybrid pairs.

The results of this portion of Experiment B indicate that, in general, opaque-2 hybrids have greater susceptibility than normal hybrids to 2nd-brood corn borers; however, this difference was primarily due to hybrid pair W64A × A632.

Inbreds with 2nd-brood infestation

At 2 weeks, there was no difference in the number of borers, tunnels or mean instar between the genotype groups (Table 5). Number of borers at 4 weeks was quite high compared to survival of the 1st brood at 4 weeks. The opaque-2 inbred group had 12% fewer borers ($P < 0.05$), and 16% fewer tunnels ($P < 0.10$) than the normal group. Inbred pair A619 was responsible for this difference with less tunnels on the opaque-2 inbred ($P < 0.01$).

Mean borer instar was similar for both genotype groups. Inbred pair W64A had a significantly highermean instar on the opaque-2 inbred ($P < 0.10$).

Significant differences were found among the 5 inbred pairs for numbers of borers and tunnels/plant and mean borer instar at 2 and 4 weeks. Inbred pairs A632 and A619 tended to be the most susceptible of the inbreds and pairs B37 and B14A tended to be the most resistant.

The results of this part of Experiment B showed no general trends of greater susceptibility of either genotype; however, inbred pair A619 showed

Table 5

Number of borers and tunnels per plant and mean borer instar at 2 and 4 weeks, for inbreds with 2nd-brood infestation

Inbreds	genotype	2 weeks			4 weeks		
		borers	tunnels	instar	borers	tunnels	instar
A619	0—2	9.0**	7.9	4.18	5.4**	8.9**	5.16
	N	13.4	9.5	4.30	8.9	14.2	5.23
A632	0—2	11.2	5.2	4.21+	6.8	6.1	5.10
	N	10.2	4.3	4.04	7.2	5.8	5.00
B14A	0—2	8.2	2.5	3.74	5.3	3.2	4.80
	N	8.8	1.9	3.80	5.5	2.1	4.91
B37	0—2	8.2*	3.2	4.89	5.4	3.0	4.88
	N	5.8	2.2	3.94	5.5	4.0	4.86
W64A	0—2	8.3	3.5	3.71	5.8	4.3	5.08+
	N	9.0	4.1	3.83	5.6	4.2	4.85
Group mean	0—2	8.98	4.46	3.95	5.75*	5.10+	5.00
	N	9.45	4.42	3.98	6.54	6.04	4.97

** 1% level of significance
* 5% level of significance
+ 10% level of significance

consistently lower borer survival, development and tunnelling on the opaque-2 genotype.

Comparison of hybrids and inbreds with 2nd-brood infestation

The opaque-2 hybrids generally had greater borer survival, development and tunnelling than the normal hybrids; however, the results for inbreds did not show this trend. Hybrid pair W64A × A632 consistently had higher borer survival, development and tunnelling on the opaque-2 hybrid. Neither inbred parent showed significant differences between genotypes for borer survival or tunnelling, however, borer development was significantly higher on both opaque-2 inbreds. Hybrid pairs A619 × A632 and W64A × A619 showed little differences between genotypes, and their inbred parent A619 had strong differences, with borer survival and tunnelling considerably lower on the opaque-2 genotype.

Hybrid pair B37 × B14A had slightly more borers at 2 weeks and slightly fewer tunnels at 4 weeks on the opaque-2 genotype. Inbred B14A had slightly fewer borers on the opaque-2 genotype, but B37 had significantly more borers on the opaque-2 genotype. Hybrid pair B37 × B14A and inbred pairs B37 and B14A generally had very low borer survival, development and tunnelling with 2nd-brood infestation.

Comparison of 1st- and 2nd-brood infestations of hybrids and inbreds

The 2nd-brood infestation of hybrids and inbreds resulted in much higher borer survival, development and tunnelling than the 1st-brood infestation. Second-brood borer survival was about 2 times higher than the 1st-brood at 2 weeks and 5 times higher at 4 weeks. This could in part be due to progress that has been made in breeding for resistance to the 1st-brood and lack of progress toward 2nd-brood resistance. Also, at the time of the 2nd-brood infestation, weather conditions may be more favourable for survival and development. Fully developed corn plants provide many protected feeding sites that aid in borer survival and rapid development. Measurable tunnelling occurred at 2 weeks with 1st-brood infestation. The greater borer development in the 2nd-brood may be one reason for earlier tunnelling. Also, the favoured feeding sites of the 2nd-brood (sheath, ligule and collar tissues) are closely associated with the plant stalk.

Hybrids responded similarly to 1st- and 2nd-brood infestations in that there were few significant differences between the genotypes of pairs. Where significant differences occurred, the opaque-2 genotypes were generally more susceptible. Hybrid pair W64A × A632, which consistently showed greater susceptibility of the opaque-2 hybrid with 2nd-brood infestation, indicated similar susceptibility with 1st-brood infestation, although the differences were not as marked.

Although differences were not significant, hybrid pair B37 B14A had lower borer survival at 2 weeks and a lower number of tunnels at 4 weeks on the opaque-2 hybrid with both 1st- and 2nd-brood infestations. This trend did not occur in Experiment A. Hybrid pair B37 × B14A also had the lowest borer survival and tunnelling of the hybrid pairs with both 1st- and 2nd-brood infestation.

There were few significant differences between genotypes among the inbreds with both 1st- and 2nd-brood infestations. With 1st-brood infestation, the opaque-2 genotype of inbred pair A619 was more susceptible than the normal genotype, and with 2nd-brood infestation the reverse was true. The normal genotype of inbred pair A619 was primarily responsible for this reverse of responses, having very low borer survival with 1st-brood infestation and the highest borer survival, development and tunnelling of all the inbreds with 2nd-brood infestation.

Experiment C: B14A in hybrid combination with first-brood infestation

Inbred B14A is of special interest since it showed the "reverse response" for 1st-brood survival (Table 3). B14A was examined in hybrid combination with B37 in Experiments A and B and no significant differences were found between the genotypes. Both genotypes of hybrid pair B37 × B14A had low numbers of borers particularly at 4 weeks, and it appeared from Experiment B that both inbred parents contributed to this resistance.

At 1 week there were 15% fewer borers on the opaque-2 group ($P < 0.05$; Table 6). Hybrid pair B37 × B14A had fewer borers at 1 week on the opaque-2 hybrid ($P < 0.01$). The other 2 hybrid pairs had slightly fewer borers on the opaque-2 hybrids, but these differences were not significant.

Table 6

Number of borers and tunnels per plant and mean borer instar for B14A hybrids with 1st-brood infestation (1973)

Hybrids	genotype	1 week		2 weeks		3 weeks		
		borers	instar	borers	instar	borers	tunnels	instar
A632 × B14A	0—2	13.6	1.54	8.3*	2.10	2.3	1.4	3.57
	N	15.4	1.63	10.6	2.06	2.1	1.3	3.46
B37 × B14A	0—2	11.1**	1.64	8.2	2.21	0.8	0.6	3.44
	N	16.0	1.53	8.6	2.14	1.2	0.8	3.19
W64A × B14A	0—2	15.1	1.70+	9.9	2.13*	1.1	1.2*	3.40
	N	15.4	1.53	9.1	1.93	0.9	0.5	3.06
Group mean	0—2	13.3*	1.63	8.9	2.15+	1.4	1.1	3.47+
	N	15.6	1.56	9.4	2.04	1.4	0.9	3.24

** 1% level of significance
* 5% level of significance
\+ 10% level of significance

At 2 and 3 weeks there were no significant differences in the number of borers for the genotype groups. There were 6% fewer borers on the opaque-2 group at 2 weeks. Hybrid pair A632 × B14A had fewer borers on the opaque-2 hybrid at 2 weeks ($P < 0.05$).

There were no tunnels at 2 weeks in any of the hybrids dissected, and at 3 weeks tunnelling had been started by some of the few remaining borers. There were no significant differences in the number of tunnels for the genotype groups, but W64A × B14A had more tunnels on the opaque-2 hybrid ($P < 0.05$).

Mean instar was higher for the opaque-2 hybrid group at each sampling period. Hybrid pair W64A × B14A had a higher mean instar at 1 and 2 weeks on the opaque-2 hybrid ($P < 0.10$ and < 0.05), and this may account for the higher number of tunnels on the opaque-2 hybrid. The slightly higher mean instar on opaque-2 genotypes was also found to be the case for inbred pair B14A and hybrid pair B37 × B14A in Experiment B with 1st-brood infestation.

Both genotypes of the B14A hybrids appear to be highly resistant with very low borer survival at 3 weeks. High mortality occurred at the time larvae began to enter the stalk during the 3rd week of feeding.

Results of Experiment C indicate that some hybrids with B14A as one of the parents do not show greater borer survival on the opaque-2 hybrids as was found with other pedigrees. The B14A hybrids tended to show the "reverse response" of lower borer survival on the opaque-2 genotype; however, this trend was found only at very early sampling periods. Borer development was found to be greater on the opaque-2 genotypes.

Conclusions

It must be concluded that opaque-2 hybrids generally have greater susceptibility to the European corn borer. WINDELS *et al.* (1972) compared commercial opaque-2 and normal hybrids for 1st-brood survival and tunnelling and found opaque-2 hybrids had 25 to 33% more borers and 18 to 54% more tunnels. In the present studies, commercial and open-pedigree opaque-2 hybrids had 17 to 26% more 1st-brood borers and 13% more tunnels than the normal hybrids. Open-pedigree opaque-2 hybrids as compared with normal hybrids had 7% more 1st-brood borers and 16% more tunnels, and borer development was 0.11 to 0.28 stadia greater. Opaque-2 inbreds had 9% more 1st-brood borers and 21% more tunnels than normal inbreds, and borer development was 0.10 stadia greater.

The degree of greater susceptibility varies with the pedigree. Hybrids with B14A as one of the parents showed the "reverse response" with 6 to 15% fewer borers on the opaque-2 genotype. Borer development was 0.07 to 0.23 stadia greater on opaque-2 B14A hybrids. The most resistant hybrid pair to 1st-brood borers was B37×B14A.

The opaque-2 hybrids with 2nd-brood infestation had 8 to 10% more borers and 16% more tunnels than the normal hybrids. Opaque-2 and normal inbred groups were similar with 2nd-brood infestation.

Greater borer survival on opaque-2 hybrids results in increased tunnelling and probably reduced yields. In addition to immediate yield losses, the number of borers overwintering or entering the next generation will probably be greater and this would contribute to additional crop losses from future generations.

Borer development was greater on opaque-2 genotypes. Advanced borer development on opaque-2 hybrids is undesirable. Advanced instars invade stalk tissue earlier, which provides protection from predators, parasites and insecticides. First-brood borers completing development earlier are more likely to pupate and contribute to the 2nd brood, causing additional crop damage and increasing borer populations. Second-brood borers with advanced development are less likely to be killed by early frosts in northern corn-growing regions such as Minnesota.

The greater susceptibility of opaque-2 hybrids can be avoided through the use of hybrids showing the "reverse response" or through the selection of opaque-2 hybrids that have lower borer survival than normal hybrids of another pedigree. Inbred B14A appears to contribute to the "reverse response" in hybrid combination and also to low borer survival. In hybrid pair B37 × B14A in particular, the opaque-2 hybrid was more resistant than its normal counterpart and all the other hybrids evaluated. Hybrids that showed no difference in susceptibility between opaque-2 and normal genotypes are also desirable.

These findings indicate some disadvantages to the general use of opaque-2 hybrids; however, through pedigree selection, these disadvantages may be minimized.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (3—4), pp. 335—346 (1976)

CYTOGENETICAL EFFECTS OF CHEMICAL AND PHYSICAL MUTAGENS ON DEVELOPING EMBRYOS OF NIGELLA DAMASCENA L.

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An original technique has been developed of treating gametes, zygotes and early embryos of *Nigella damascena* L. with chemical and physical mutagens. The delay in fertilization and the slowing down of the rate of cell division in the embryo and endosperm after mutagen effect have been found. The induction of chromosome aberrations proceeds for at least 80 hours after chemical mutagen treatment, while after irradiation no new chromosome aberrations appear. A positive correlation between the number of chromosome aberrations and the depression of plant growth and development has been observed. The frequency of cells with chromosome aberrations manifested in AI of meiosis significantly increases after mutagen treatment. As a result the amount of sterile pollen increases and plant fertility decreases.

Introduction

Recently several attempts were made to decode the nature and mechanism of mutagenesis specificity (ref.). The specificity of mutagenesis is related to processes between the moment of mutagen exposure up to the registration of mutations induced. These stages are as follows: the mutagen penetrates into the cell, is transported to the gene, comes into contact with the gene and reacts with it. The primary reaction must be released being subjected to selection pressure. A number of processes occurring at each of these stages provide a characteristic pattern of mutagenesis (SAHAROV 1932, RAPAPORT 1947, 1966, OGANESYAN 1969).

Studies on artificial mutagenesis are usually carried out on dry or swollen seeds, i.e. a completely developed multicellular embryo is subjected to mutagens. Only the mutations appearing in a few initial cells which afterwards form generative tissues may be realized during subsequent seed generations. The same factor determines specific numerical relations between the mutants in M_2.

Studies of mutagen effect on the embryo at early stages of ontogenesis have been published (MERICLE—MERICLE 1961a, b, EEVREUX—SCARASCIA—MYGNOZZA 1962, TRAMVALIDIS—DEVREUX 1964, MONTI 1967, BHADURI — SHOME 1969). The authors used physical factors and showed that ionizing irradiation of the early stages of ontogenesis produced the largest number of highly fertile mutants.

To reveal the specificity of the mutation process after treatment of the early stages of embroyogenesis, we applied both physical and highly active chemical mutagens.

It could be expected that peculiarities of the structure of embryos and of their functions during early ontogenesis as compared to those of resting seeds would lead to essential differences in mutagen ability to penetrate through the cell membranes and provide better conditions for the interaction of the mutagen with the cell genome. Thus not only the frequency but also the spectrum of the mutations would be changed. The fact that an embryo consists of few cells at this developmental stage should promote the realization in M_2 of the majority of initiated mutations.

Material and Method

Nigella damascena L. *(Ranunculaceae)* was used. It has 6 pairs of chromosomes which are well distinguished morphologically. It is also a convenient object for cytological studies and is often used for solving various cytogenetic problems. It is also convenient for genetic purposes.

X-irradiation (Xr) and 4 chemical mutagens (ethilenimine (EI), nitrozomethylurea (NMU), diethylsulphate (DES) and trimethylphosphate (TMP) were used. Air-dried seeds, gametes, zygotes and embryos at early developmental stages were treated with mutagens (Table 1).

120—140 plants for each variant were grown in a greenhouse. Only flowers of the main stem of M_0 plants of equal age were treated. In the experiments with EI and TMP, 140 flowers were treated in each varient, with NMU — 100 flowers, and with DES — 85 flowers. In the greenhouse 20—40 seedbuds were usually formed in each boll of the M_0 plants and only in single plants they numbered up to 80.

The treatment with chemical mutagens was performed immediately after pollination: treatment was carried out in a special chamber made of organic glass. Chemical mutagens (5 ml) were injected with a syringe into each boll with seedbuds and washed out with water and 5% solution of sodium hyposulphite.

In each series of radiation experiments 115 plants were irradiated on RUP — 200, 4 hrs after pollination of the M_0 plants. A special experiment was run for comparison in which air-dried seeds were treated with aqueous solutions of mutagens. 100 plants of M_0 were grown for the control.

Immediately after pollination the flowers were fixed in Navashin solution at 5 min intervals for one hour to study the rate of pollen germination and its penetration into the embryonic sac.

To determine the stages of embryonic development ovules with seedbuds were fixed in Navashin solution every 2 hrs for 20 hrs after mutagen treatment.

Cytoembryological studies were carried out on constant preparations. Longitudinal sections of seedbuds were stained with haematoxylin according to Heidenhain. Accelerated staining with iron haematoxylin was also applied.

To perform cytological analyses of induced chromosome aberrations in germ cells the material was fixed in acetic acid-alcohol (3 : 1) immediately after the treatment with mutagens, every 2 hrs for 80 hrs.

To determine the frequency of chromosome aberrations induced during the treatment of air-dried seeds a portion of seeds was germinated at 20—22 °C. Fixation was carried out every two hours beginning at 25 hrs after moistening, up to 80 hrs.

Table 1 shows the data on ovary formation in M_0 after treating seedbuds with various mutagens. Depending on ovary formation, different amounts of seed were taken in different experimental series while sowing M_1 seeds.

In the experiment on treating air-dried seeds with mutagens 500 seeds in each variant were sown in M_1. 500 seeds were also sown in the control.

The effect of mutagens on plant growth and development was carefully studied in M_1. To perform a cytological analysis of the meiosis of M_1 plants, 30 buds from each variant were fixed in acetic alcohol during budding.

Table 1

Effect of mutagens on germination of seeds after the treatment of early stages of embryonic development of Nigella damascena L. (scheme and volume of the experiment)

Material treated	Mutagen	Variant	Dose (r) concentration (%)	Exposition (hrs)	Number of treated seedbuds	Seeds obtained	
						Number	%
1	2	3	4	5	6	7	8
—	—	Control (1)	—	—	1 385	974	70.32 ± 1.22
Gametes, zygotes, proembryos	X-irradiation	Xr$_1$	300	—	1 715	639	37.26 ± 1.16
		Xr$_2$	600	—	1 638	617	37.66 ± 1.20
		Xr$_3$	900	—	1 724	548	31.78 ± 1.11
		Xr$_4$	1 200	—	1 419	375	26.42 ± 1.17
		Mean value			6 496	2 179	33.54 ± 0.58
	Ethilen-imine	EI$_1$	0.003	8	1 165	647	55.53 ± 1.45
		EI$_2$	0.005	8	1 216	615	50.57 ± 1.43
		EI$_3$	0.008	8	1 468	523	32.62 ± 1.19
		EI$_4$	0.003	12	1 345	558	41.48 ± 1.34
		EI$_5$	0,005	12	1 625	617	37,96 ± 1,20
		EI$_6$	0,008	16	1 514	474	31,30 ± 1,19
		EI$_7$	0,003	16	1 395	468	33,55 ± 1,26
		EI$_8$	0,005	16	1 685	346	20,53 ± 0,98
		EI$_9$	0,008	16	1 842	325	17,64 ± 0,88
		Mean value			13 255	4 573	34,50 ± 0,41
	Nitrozomethylurea	NMU$_1$	0,008	8	1 275	462	36,23 ± 1,34
		NMU$_2$	0,01	8	1 139	418	36,69 ± 1,42
		NMU$_3$	0,005	12	1 315	425	32,31 ± 1,29
		NMU$_4$	0,008	12	1 428	387	27,10 ± 1,17
		NMU$_5$	0,01	12	1 359	341	25,09 ± 1,17
		NMU$_6$	0,005	16	1 576	384	24,36 ± 1,13
		NMU$_7$	0,008	16	1 558	386	24,77 ± 1,09
		NMU$_8$	0,01	16	1 611	364	22,59 ± 1,03
		Mean value			11 261	3 167	28,12 ± 0,42
	Diethylsulphate	DES$_1$	0,05	8	1 167	715	61,26 ± 1,42
		DES$_2$	0,1	8	1 255	687	54,74 ± 1,40
		DES$_2$	0,05	12	1 345	589	43,79 ± 1,35
		DES$_4$	0.1	12	1 264	501	39.63 ± 1.37
		DES$_5$	0.05	16	1 057	437	41.34 ± 1.51
		DES$_6$	0.1	16	1 418	425	29.97 ± 1.21
		Mean value			7 506	3 354	44.68 ± 0.56

Table 1 (continued)

Material treated	Mutagen	Variant	Dose (r) concentration (%)	Exposition (hrs)	Number of treated seedbuds	Seeds obtained	
						Number	%
1	2	3	4	5	6	7	8
Gametes, zygotes, proembryos	Trimethyl-phosphate	TMP_1	0.01	8	1 427	235	16.46 ± 0.98
		TMP_2	0.01	12	1 589	287	18.06 ± 0.96
		TMP_3	0.01	16	1 705	218	12.78 ± 0.80
		Mean value			4 721	740	15.67 ± 0.52
Air-dried seeds	X-irradiation	$Xr-DS_1$	9.000	—	—	—	—
		$Xr-DS_2$	12.000	—	—	—	—
	Ethilen-imine	$EI-DS_3$	0.025	12	—	—	—
		$EI-DS_4$	0.025	18	—	—	—
		$EI-DS_5$	0.050	12	—	—	—
		$EI-DS_6$	0.050	18	—	—	—

(1) Normal seedbuds without treatment

Results

Our studies showed that *Nigella damascena* L. had unicellular archespore, an embryonic sac of the standard type with eight nuclei, three large unicellular antipodes and a small egg cell.

When the weather was favourable *Nigella* pollen started germination on the stigma 10 min after pollination. 35—40 min after pollination the pollen tubes reached the style end and entered the ovary. 50 min after the entry of the spermia into the embryonic sac their fusion with the egg cell and secondary nucleus was observed (Fig. 1). Additional sperm cells (3—8 instead of 2) were often observed in the same embryonic sac. After fertilization a short resting period was seen (Fig. 2), then 3 hrs after pollination the secondary nucleus was divided, initiating the endosperm development. After 4 hrs the two-cell embryo and binucleate endosperm were formed. After 6 hrs the four-cell embryo and tetranucleate endosperm were formed. 7 hrs after pollination the division of the embryo and endosperm became more intensive and after 10—12 hrs the multicellular embryo and endosperm were observed. After two days seed-lobes began to show in the embryo. After 6 days radicle, root-cap and plumule were seen.

This is the normal course of development of *Nigella damascena* L. under green-house conditions at 25—28 °C. The change of environment results in a disturbance of normal development.

It was found that chemical mutagens drastically depressed fertilization and the rate of embryonic development (Fig. 3). The strongest effect was produced by EI and TMP, particularly at high doses (Fig. 4). Unfertilized egg-cells were observed up to 12 hrs after pollination, while multicellular embryos (more than 8 cells) appeared only 20 hrs after pollination. Many seedbuds of the plants of these variants died at different developmental stages. The least pronounced effect was produced by DES: in its presence the developmental rate became slower only by 2–4 hrs as compared with the control. NMU and X-irradiation produced intermediate effect.

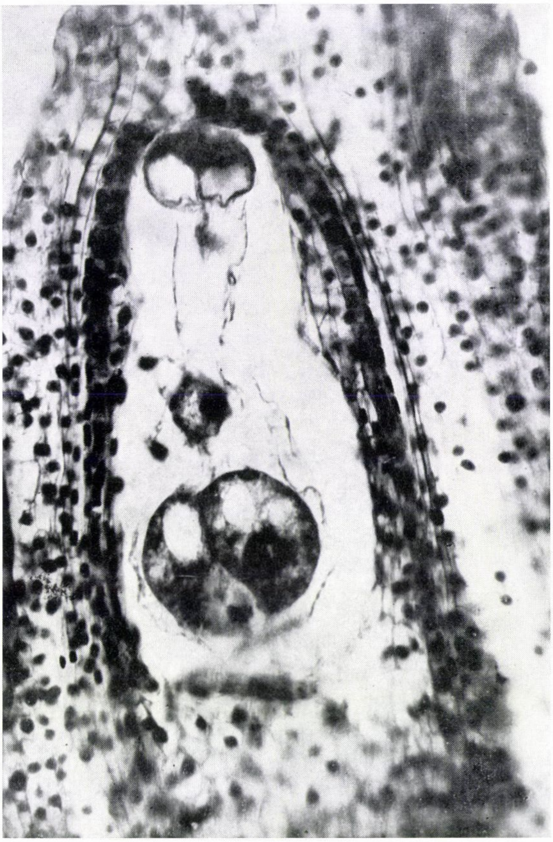

Fig. 1. Embryonic sac of *Nigella damascena* L. in the moment of mutagen treatment. 1. before fertilization; 2. 2 hrs after fertilization

Thus our procedure at low exposure (8 hrs) provided mutagen-treatment of gametes, zygotes and of the first developmental stages of the embryo (at low concentrations of mutagens). Under 16 hrs exposure mutations should also be induced in a relatively multicellular embryo. As X-irradiation was applied

4 hours after pollination mainly fertilized egg-cells and two cell embryos were treated.

It is known that the induction of mutations proceeds for rather a long time after the exposure to chemical mutagens (DUBININ—SAPRIKINA 1964, SIDOROV—SOKOLOV—ANDREEV 1965). In this connection we decided to check the duration of mutation induction after treating seed embryos with the mutagens. Chromosome aberrations were used as a criterion. The material was analysed for 80 hrs after the beginning of treatment. The mutagen effect brought about a significant retardation in the onset of mitosis (at large doses of EI and TMP especially) and prolonged the mitotic cycle.

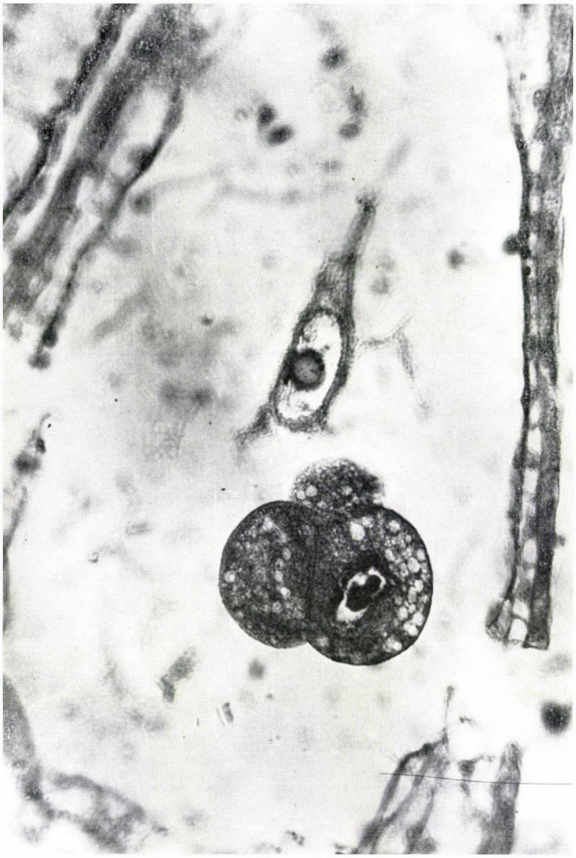

Fig. 2. Embrionic sac of *Nigella damascena* L. in the moment of mutagen treatment. 1. before fertilization: 2. 2 hrs after fertilization

The maximum number of cells with aberrations was observed at 10—14 hrs after irradiation, and 22—28 hrs after the treatment with chemical mutagens.

The mutation process practically stopped 40 hrs after irradiation. New aberrations of chromosomes (of a chromatid type) were observed after treatment with chemical mutagens up to the end of fixation, i.e. up to 80 hrs. Consequently in this case induced mutations occurred in almost completely formed embryos.

Fig. 3. Embryonic sac 10 hrs after treatment with low concentration of ethylenimine

As indicated in Table 2, a large number of chromosome aberrations are found in embryonic cells after the irradiation of zygotes and proembryos (max. at Xr_4 — 23.6%). High doses of chemical mutagens induce almost the same quantity of chromosome aberrations as irradiation (max. TMP_2—23.7%; EI_7 — 22.0%; NMU_6 19.0%; DES — 16.3%). At the same time, on average, all the chemical mutagens studied, except TMP, turned out to be 1.5—2 times less effective. When dry seeds were treated, a higher aberration frequency was observed in both cases. The efficiency of these mutagens was practically the same.

Table 2

Cytogenetic effect of radiation and chemical mutagens on Nigella damascena L.

Material treated	Variant	Chromosome aberrations in mitosis		Chromosome aberrations in meiosis		
		Number of cells studied	Maximum frequency of cells with chromosome aberrations (%)	Number of cells studied	Number of cells (%) with	
					Chromosome bridges	Micronuclei
1	2	3	4	5	6	7
Gametes, zygotes, proembryos	Control	237	1.26 ± 1.12	118	—	0.86 ± 0.82
	Xr_1	102	16.67 ± 3.69	100	1.00 ± 0.99	1.00 ± 0.99
	Xr_2	102	15.69 ± 3.60	115	2.60 ± 1.58	2.60 ± 1.58
	Xr_3	194	21.65 ± 2.96	130	4.61 ± 1.91	3.85 ± 1.68
	Xr_4	93	23.65 ± 4.40	120	6.66 ± 2.28	5.83 ± 2.16
	Mean value	491	19.75 ± 1.79	465	4.30 ± 0.94	3.41 ± 1.85
	EI_1	204	3.43 ± 1.27	56	—	—
	EI_2	228	9.64 ± 1.95	38	—	—
	EI_3	203	9.85 ± 2.09	31	—	—
	EI_4	214	14.0 ± 2.37	115	0.86 ± 0.82	0.86 ± 0.82
	EI_5	247	17.0 ± 2.39	100	1.00 ± 0.99	1.00 ± 0.99
	EI_6	249	17.67 ± 2.42	115	0.86 ± 0.82	1.73 ± 1.33
	EI_7	245	22.04 ± 2.65	115	1.73 ± 1.33	1.73 ± 1.33
	EI_8	—	Strong disturbance of cell division			
	EI_9	—	The same	130	3.07 ± 1.75	4.61 ± 1.91
	Mean value	1590	13.77 ± 0.86	800	1.50 ± 0.42	1.87 ± 0.47
	NMU_1	—	—	79	—	—
	NMU_2	402	5.47 ± 1.32	71	—	—
	NMU_4	410	9.76 ± 1.46	—	—	—
	NMU_5	409	13.48-± 1.69	106	0.94 ± 0.96	0.94 ± 0.96
	NMU_6	242	19.01 ± 2.52	115	1.73 ± 1.33	0.86 ± 0.82
	NMU_7	—	Strong disturbance of cell division			
	NMU_8	—	The same	115	2.60 ± 1.58	2.60 ± 1.58
	Mean value	1463	11.14 ± 0.82	601	1.49 ± 0.49	1.16 ± 0.43
	DES_3	226	3.98 ± 1.30	—	—	—
	DES_4	207	8.69 ± 1.96	—	—	—
	DES_5	210	14.28 ± 2.41	—	—	—
	DES_6	214	16.35 ± 2.53	—	—	—
	Mean value	875	10.51 ± 1.03	—	—	—

Thus it was stated that the mutagens under study produced a strong effect on the rate of embryo development, moreover, mutations were induced by chemical mutagens for a long time after the cessation of treatment.

The treatment with mutagens induced essential changes in development and growth and caused a significant decrease in the fertility of M_1 plants.

Table 2 (continued)

Material treated	Variant	Chromosome aberrations in mitosis		Chromosome aberrations in meiosis		
		Number of cells studied	Maximum frequency of cells with chromosome aberrations (%)	Number of cells studied	Number of cells (%) with	
					Chromosome bridges	Micronuclei
1	2	3	4	5	6	7
Gametes, zygotes, proembryos	TMP_1	282	13.47 ± 2.03	115	0.86 ± 0.82	1.73 ± 1.33
	TMP_2	304	23.68 ± 2.44	115	2.60 ± 1.58	2.60 ± 1.58
	TMP_3	—	Strong disturbance of cell division	130	5.38 ± 2.08	5.38 ± 2.08
	Mean value	586	18.77 ± 1.61	360	3.05 ± 0.90	3.33 ± 0.94
Air-dried seeds	$Xr-DS_1$	238	21.00 ± 2.64	100	4.00 ± 1.95	4.00 ± 1.95
	$Xr-DS_2$	242	30.16 ± 2.95	100	10.00 ± 3.00	8.00 ± 2.71
	Mean value	480	25.62 ± 1.99	200	7.0 ± 1.80	6.0 ± 1.68
	$EI-DS_3$	214	12.14 ± 2.23	100	2.00 ± 1.40	1.00 ± 0.99
	$EI-DS_4$	237	19.83 ± 2.59	100	4.00 ± 1.95	2.00 ± 1.40
	$EI-DS_5$	224	23.66 ± 2.84	100	6.00 ± 2.37	4.00 ± 1.95
	$EI-DS_6$	211	29.38 ± 3.13	100	6.00 ± 2.37	6.00 ± 2.37
	Mean value	886	21.21 ± 1.37	400	4.5 ± 1.03	3.25 ± 0.88

Variants with the highest percentage of chromosome aberrations in the cells of seedbuds and radicle meristem showed a sharp decrease in plant germination and survival rate, as well as a strong inhibition of growth.

The analysis of meiosis in M_1 plants showed that after mutagen treatment a significant proportion of the induced chromosome aberrations (probably, symmetrical translocations and inversion primarily) was preserved up to this developmental stage and could be found in anaphase I. An especially large number of aberrations are found in anaphase I after X-irradiation of the early developmental stages and of dry seeds in particular. Of the chemical mutagens, TMP turned out to be the most effective in inducing large chromosome aberrations preserved up to AI, while treatment with the highest doses of NMU induced a small percentage of cells with aberrations in anaphase I.

While comparing the results of EI action on the embryos at early developmental stages and on dry seeds, it was revealed that in the latter case more cells with aberrations were preserved up to anaphase I of meiosis.

Comparison of the mean frequency of cells with chromosome aberrations during the first mitosis with the fertility of M_1 plants points to a close relationship between these processes.

Fig. 4. Embryonic sac 18 hrs after treatment with high concentration of ethylenimine and trimethylphosphate

The treatment of gametes, zygotes and proembryos with EI and NMU showed that the values of maximum frequency of cells with chromosome aberrations were close (Table 2). The fertility of plants was also in close relation with these results. DES turned out to be less effective in inducing chromosome aberrations, and the highest fertility was also observed in these variants. TMP treatment induced the maximum frequency of cells with aberrations, which was parallelled by minimum fertility (Table 1).

Hence a distinct relationship was found between the frequency of mutagen-induced chromosome aberrations and peculiarities of growth, development and fertility of M_1 plants.

The mutagen effect was also manifested in the number of changed M_1 plants. EI treatment of the embryos at early developmental stages turned out to be most effective (EI_9—20.8% ± 3.19%). The number of radiomorphoses in this case was somewhat smaller (Xr_4 — 16,96% ± 2.89%).

Treatment of dry seeds with the maximum dose of EI induced more changes than irradiation (15.55 ± 3.11% and 9.39±3.06%, respectively).

After the treatment of early developmental stages, 27 types of change were observed in M_1. Most of them were manifested in variations of shape and colour of the leaves, stems and flowers. EI induced the largest spectrum of such changes. According to the capacity of inducing changes in M_1 plants the mutagens applied in our study can be arranged as follows:

$$\text{EI} > \text{NMU} > \text{DES} > \text{TMP} > \text{Xr}$$

After mutagen treatment on dry seeds the spectrum of changes in M_1 plants was very narrow. Only 9 types of morphological change were found in all the variants of this experiment.

The occurrence of plants with two, three and even four changed traits was observed. Changes in M_1 usually appeared as chimeric structures. With the increase of mutagen dose the number of chimeric plants decreased. In other words, with an increase of mutagen dose the number of completely changed plants increased, but when the doses were small, chimeric plants were more often observed. It should be noted that after the treatment of early developmental stages the frequency of chimeric plants turned out to be about 3 times lower than that in the experiments on dry seeds (13.74 ± 2.13% and 42.51 ± 4.38%, respectively).

Thus, the results of the present work show that the action of mutagens immediately after pollination brings about the retardation of fertilization and the rate of cell division of the embryo and endosperm. The strongest effect is produced by large doses of TMP and EI. The mutation process in the embryonic cells proceeds for at least three days after washing away the chemical mutagens. In the variants with the largest percentage of chromosome aberrations in the seedbud cells and radicle meristem a sharp decrease of germination and survival and a strong depression of growth are observed. In treated plants the frequency of chromosome aberrations significantly increases in anaphase I of meiosis as compared to the control, especially after X-radiation. As a result the amount of sterile pollen increases and the fertility of M_1 plants decreases.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (3–4), pp. 347–363 (1976)

THE FLUORESCENCE EVIDENCE OF THE INTERACTION OF MYOSIN SUBSTRATE (ATP) WITH HISTIDINES AND OTHER BASIC AMINO ACIDS

By

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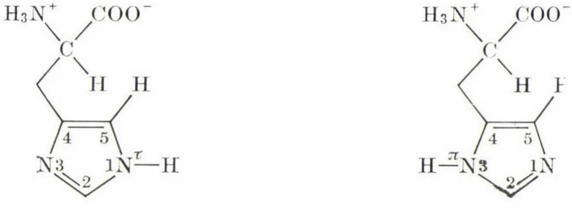
The interaction of the contact amino acids, which play a role in the enzymatic function of ATP, myosin (His, MeHis, Lys, Me_3Lys, Cys), and the formation of their chelates have been investigated by spectrofluorometry. It has been established that His, MeHis, Me_3Lys and Cys interact with ATP and form chelates in the presence of Mg^{2+}, while they cause a considerable quenching of the fluorescence of ATP. Lys alone does not interact with ATP and moderates the quenching of fluorescence by the other amino acids. In the course of the interaction of ATP—Me_3Lys, reactive phosphoryl cation is liberated, which actually means that ADP is interacting with Me_3Lys. It is assumed by the authors that the phosphoryl cation is bound by MeHis, and that the preferred histidines of myosin are phosphorylated through this intermediate. An important role is attributed to cysteines of various reactivity in the binding of the substrate and particularly in the initiating of two reaction routes.

Introduction

The participation of histidine in several enzyme reactions has been pointed out earlier (Abduhl-Fadl—King 1949, Cohn—Edsall 1943, Morton 1956, Bergmann *et al.* 1956, Rüterjans—Witzel 1969). A summary of early literature was given by Joshi et al. in 1965 (Joshi *et al.* 1965). The many-sided biological role of histidine is explained in each work.

According to the IUPAC Convention (1975), the numbering of the imidazole ring of histidine begins with the N^{τ}-(tele)atom, farthest from the alanine side chain.*



I

II

Histidine tautomers

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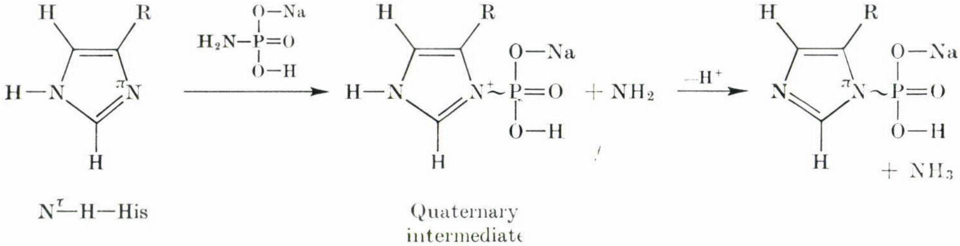
* The previous usage in biochemistry was to begin the numbering with the N^{π}-atom adjacent to the alanine side chain.

The imidazole ring occurs in two tautomeric forms, the N^{τ}—H (or 1—N—H . . .I) and the N^{π}—H (or 3—N—H . . . II) types.

According to the investigations of REYNOLDS *et al.* (1973), in neutral solutions form I is predominant, with a tautomeric ratio of 4 : 1 under physioogical pH conditions.



The participation of histidine in the enzymatic activity of myosin was assumed or observed earlier (HOTTA—MORALES 1962, STRACHER—CHAN 1964, MESHKOVA 1967, HOTTA—TERRAI 1965). The fundamental observations are summarized by BOYER (1970), who called attention to the fact that the histidine is phosphorylated in several enzymatic processes. FAZEKAS—SZÉKESSY-HERMANN (1974) reported that a substantial quantity of histidine phosphate can be isolated from myosin after alkaline hydrolysis. Therefore the present communication deals with the interaction of histidine and its derivatives, assuming that similar chelates are formed with the substrate during the enzymatic reaction of myosin.

Materials and Methods

L-1-methylhistidine,* L-3-methylhistidine** and N^{ε}-methyl-L-lysine were supplied by Serva, Heidelberg Corp. N^{ε}-trimethyl-L-lysine (TMD***) was obtained from Dr. Tyihák and has been prepared synthetically by the method of PUSKÁS—TYIHÁK (1969). The other chemicals were produced from the firms Reanal and Chinoin, Budapest. The interaction of histidine and its derivatives with ATP in the presence or absence of Mg ions has been investigated and the effect or fluorescence quenching effect of the single amino acids on ATP has been followed with a spectrofluorometer.

Fluorescence methods. Fluorescence measurements were made with a Hitachi MPF-2 A spectrofluorometer, using the conventional optical system for the detection of fluorescence at 90° relative to the path of the exciting light. A quartz cuvette, 1 cm in length was used. Samples containing ATP were excited by irradiation at a wavelength of 300 nm, except for samples containing amino acids, which were excited at the suitable wavelength as given in the experiments.***** The spectra are uncorrected.

Histidine phosphates have been prepared by synthesis. The simplest histidine phosphate-synthesis is according to RATHLEV—ROSENBERG (1956) with aminophosphoric acid monosodium

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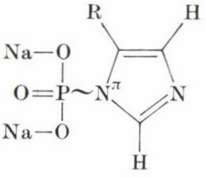
* N^{π} and ** N^{τ}—Me—His

*** Me_3Lys or TML are used alternatively for N^{ε}-trimethyl-L-lysine in this paper.

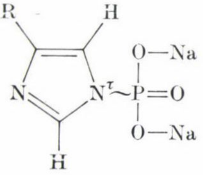
**** Distilled water used for the measurement of the fluorescence spectra exhibited second-order scattering in the vicinity of 350 nm of the Raman spectrum, at a slit width of 5, half a division of fluorescence.

salt ($NH_2—PO_3NaH$), and an oriented synthesis is realized by Hultquist *et al.* (1966) and by Hultquist (1968), by the appropriate selection of the histidine and phosphoamidate concentrations.

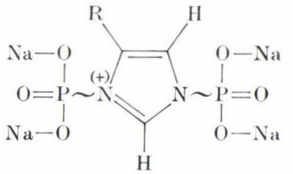
Using these methods, for the preparation of N^{π}-phosphohistidine a mixture of 0.025 M histidine and 0.33 M phosphoamidate was reacted for 1 hour at room temperature and pH 8. Since N^{π}-phosphohistidine formation reaches a maximum at 25 °C (Hultquist 1968), the reaction was stabilized by adjusting the pH to a value above 9, to prevent the rearrangement of N^{π}—P—His to N^{τ}—P—His. The mixture was then stored in a refrigerator until the preparation of the crystalline product or until the chromatographic separation of the isomers. At the beginning of the reaction, the phosphoryl cation attacks as an electrophilic substituent at the 3-N^{π}-electron-dense position of the imidazole ring.



N^{π}-P-His
(1-P-His)**



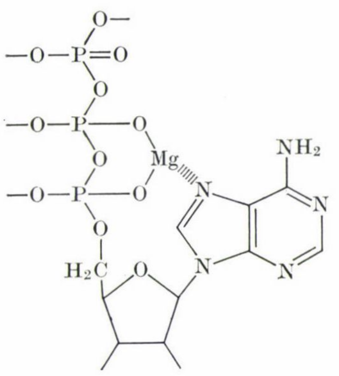
N^{τ}-P-His
(3-P-His)**



N,N-di-P-His
(1,3-di-P-His)

For the preparation of N^{τ}—P—His, a mixture containing 0.25 M histidine and 0.5 M phosphoamide was reacted at pH 8 for 2 days at room temperature, with occasional stirring. The product was then separated by chromatography, or precipitated directly in the form of the crystalline Ca salt.

According to Hultquist (1968), the method of Rathlev—Rosenberg (1966) also yields primarily N^{π}—P—His, at pH 7 in addition to a small amount of 1,3-di-P-His and a small amount of α-1,3-tri-di-P-His.

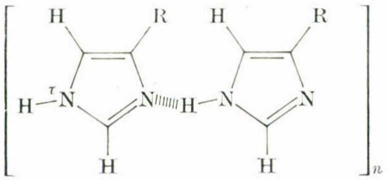


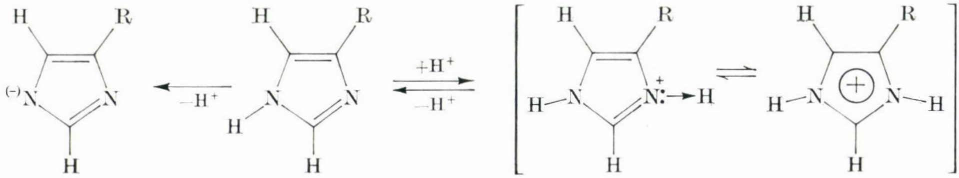
ATP—Mg-chelate

Results

In the interpretation of the biological role of myosin it has been assumed that ATP is involved in the formation of high-energy bond N-phosphates of some histidines in the polypeptide chain of the head part of myosin (Fazekas *et al.* 1976). In our opinion the hydrolytic splitting of ATP by myosin can only be observed in vitro. We consider, with other authors, that the participation of ATP in the activity of myosin occurs in the form of the Mg-chelate (Bagshaw—Trentham 1974). The ATP-Mg-chelate is of the type in which the purine N^7 atom is linked by a dative bond to the metal ion and γ-phosphate remains free (Boyer 1970). This form seems to be suitable for the realization of the transfer reactions.

Neither the formation of the Mg chelates of histidine and of the methylated amino acids occurring in myosin, nor their interaction with ATP have been investigated. Therefore, we wished to deal with the problem of, wether in vitro metal chelates are actually formed in the interaction of ATP, Mg, His, 1-Me-His*, 3-Me-His*, Lys, TML and Cys.





While studying this problem, attention was centred mainly on the formation of the ATP chelates, because the intermediate formed during the reaction of ATP and myosin is accompanied by a decrease in fluorescence intensity (Bagshaw—Trentham 1974).

ATP exhibits a narrow excitation maximum at 300 nm. Therefore, reaction mixtures containing ATP were always excited at 300 nm.

In the solutions which made up the reaction mixtures the concentration of each component was 20 mM, so that after mixing the end concentration of each of the components was 5 μmole/ml (with the exeption of mixtures containing Me-His, where the end concentration of each component was 1.25 μmole/ml).

Fig. 1 shows the fluorescence spectrum of a 5 μmole ATP solution (a), together with the effect of 5 μmole/ml of Mg ions (b) and of Mg ion + histidine (c) on the fluorescence intensity of ATP. It can be seen that the Mg ions decrease the fluorescence of ATP. Taking the fluorescence value of ATP at the

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* To facilitate the comparison of the results the biochemical terminology has been maintained. Thus, the 3-Me-His occurring in proteins has a Me-group in the N^τ-position.

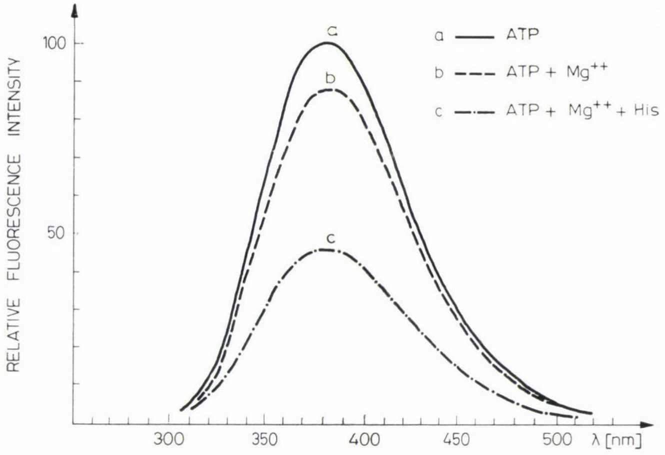


Fig. 1. Effect of Mg^{++} and Mg^{++}+His on the fluorescence spectra of ATP. Excitation at 300 nm

intensity maximum (380 nm) as 100, the Mg ions + histidine decrease the fluorescence from 100 to 46.

On excitation a 5 μmole/ml histidine solution shows an emission of much lower intensity, only about 2—3% of that of an ATP solution of the same concentration. Fig. 2 compares the fluorescence spectra of His (a), 1-Me-His (b) and 3-Me-His (c).

The spectra of both methylated derivatives differ from the spectrum of normal His, the most conspicuous being the spectrum of 3-Me-His, which extends to the region of longer wavelengths. (Owing to the low intensity, the

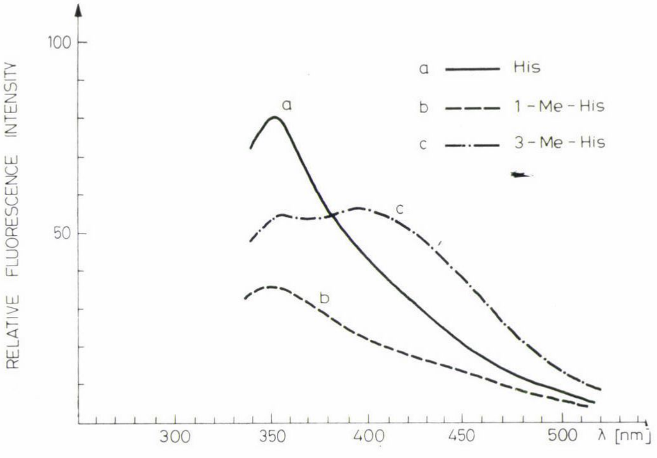


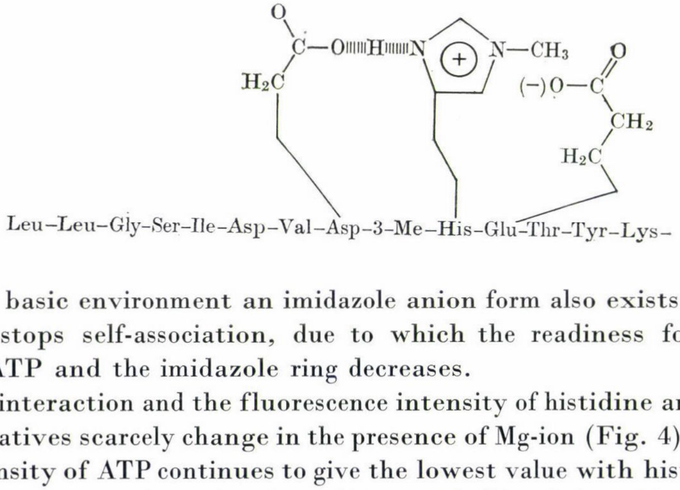
Fig. 2. Fluorescence spectra of His and N-monomethylised histidines at a concentration of 5 μmole/ml. Excitation at 290 nm

spectra of histidine and its derivatives can easily be observed by increasing the sensitivity of the instrument, but this means that at about 350 nm the Raman scattering of water also contributes to the formation of the peak. Thus, the appearance of the peak at about 350 nm can be attributed partly to Raman scattering.)

The Mg ions affect the fluorescence of His and the Me-histidines; the intensity of the fluorescence of all three amino acids is increased by the Mg ions. With histidine, the increase in intensity is scarcely 10%, but the intensity of 1-Me-His is increased by 25—27%, and that of 3-Me-His by 35%.

The effect of His and the Me-histidines on the fluorescence of ATP (Fig. 3). Even in the absence of Mg ions, there is a strong interaction between ATP and histidine, because the fluorescence emission of ATP decreases by 7—30% (b)*. 1-Me-His decreases the fluorescence intensity of ATP to about a quarter, and 3-Me-His to about half its original value (c, d).

An explanation can easily be given on consideration of the properties of the imidazole ring of histidine. Imidazoles (also histidines) have a strong tendency to interact and are present in an associated state in their solutions. This explains that the boiling point of imidazole and some of its derivatives is higher than expected. Histidine becomes protonated by stronger acids in the lower pH range and forms quaternary imidazolinum cations (marked with the limit structure).



In a basic environment an imidazole anion form also exists. Methylation probably stops self-association, due to which the readiness for interaction between ATP and the imidazole ring decreases.

The interaction and the fluorescence intensity of histidine and its methylated derivatives scarcely change in the presence of Mg-ion (Fig. 4). The fluorescence intensity of ATP continues to give the lowest value with histidine (changing the fluorescence intensity from 100 to 7% (b), while 1-Me-His lowers it from 100 to 37% (c) and 3-Me-His from 100 to 62%). On comparing the

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* The His-ATP interaction is very strongly pH-dependent and the fluorescence intensity decreases with increasing H ion concentration, and the protonated ring of adenosine in ATP has maximum fluorescence (WATENABE *et al.* 1963). Me-histidines are scarcely sensitive to pH changes.

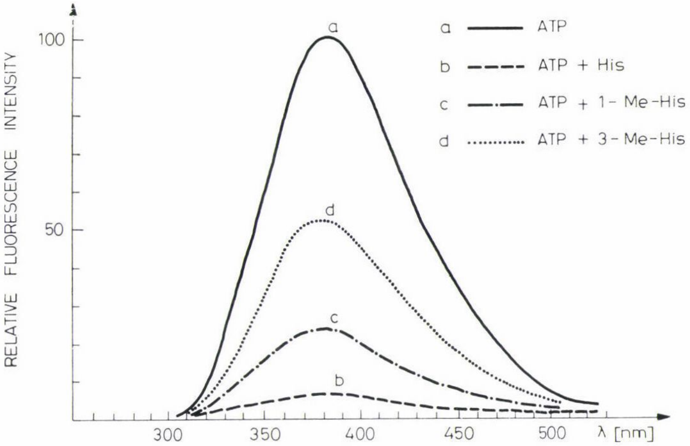


Fig. 3. **Effect of His and N-Me-histidines on the fluorescence intensity of ATP at a concentration of 1.25 μmole/ml. Excitation at 300 nm**

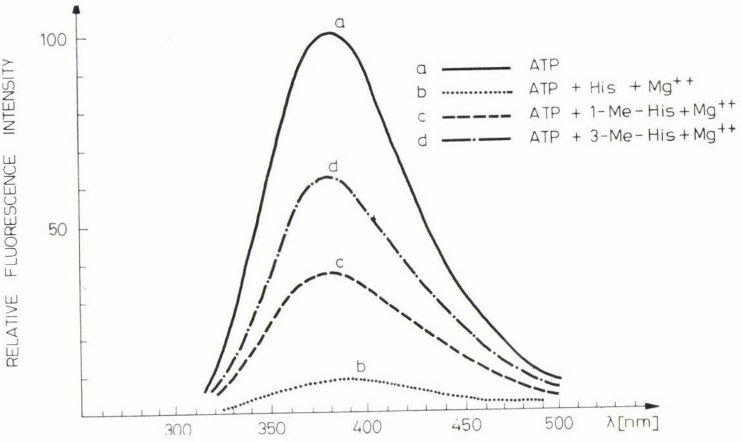


Fig. 4. **Effect of His and N-Me-histidines on the intensity of fluorescence spectra of ATP in the presence of Mg^{++} ion. The concentration of components was 1.25 μmole in all cases. Excitation at 300 nm**

fluorescence intensity of histidine in the preceding figure and in the presence of Mg ions in Fig. 4, it can be seen that the Mg ions reduce thef luorescence-quenching effect of histidine. Thus, the interaction of ATP with histidine and its methyl derivatives is stronger, while in the presence of Mg it becomes weaker due to chelate formation.

The fluorescence of lysine is very weak. It is excited at 310 nm, and exhibits a maximum at 362 nm. In our opinion at a suitable concentration the self-association of a lysine twin ion can be perceived. Such concentration-dependent spectra are shown in Fig 5 for concentrations of 5 and 20 μmole/ml.

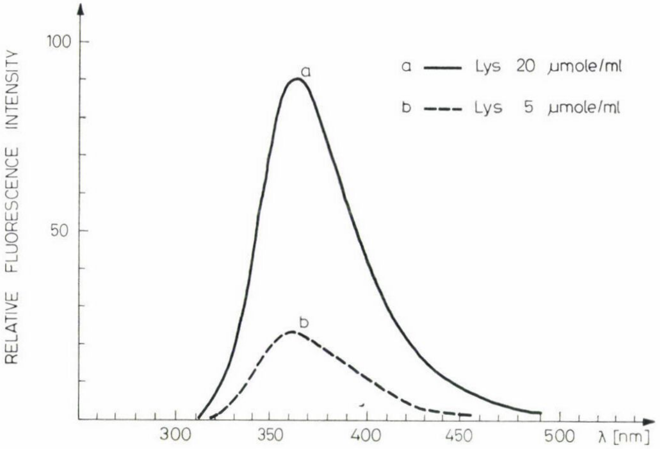


Fig. 5. **Fluorescence spectra of lysine in two different concentrations. Excitation at 300 nm**

In the spectrum of lysine, Mg ions produce only a slight change in the fluorescence, but together with histidine, in the His, Mg, Lys mixture, the fluorescence is considerably reduced. This latter shows that chelate formation occurs between the two amino acids Lys and His (Fig. 6).

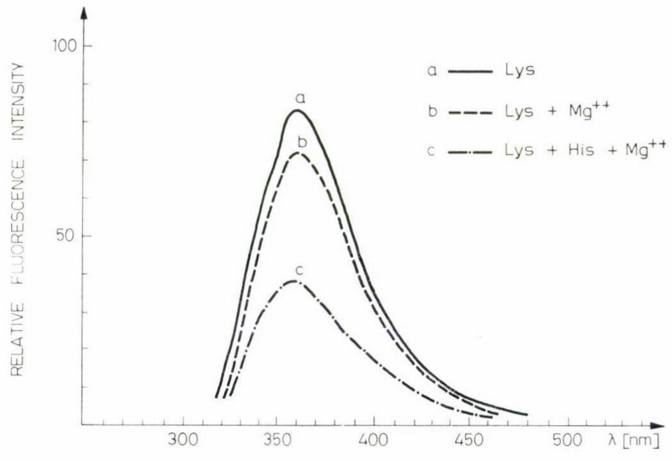


Fig. 6. **Effect of Mg^{++} and Mg^{++}+His on the fluorescence intensity of lysine at a concentration of 5 μmole/ml**

Lysine alone does not reduce, but slightly increases the fluorescence of ATP (from 100 to 107). We consider that there is no interaction between the two components, so that the fluorescence intensity is nearly additive. The addition of Mg ions to the ATP-Lys mixture indicates only a slight interaction, decreasing the fluorescence intensity of the mixture from 100 to 86.

The intensity decreases strongly, from 100 to 32, when His is also present in the mixture, and the decrease in intensity is reduced further, from 100 to 28, if all the four components (ATP, His, Lys, Mg) are present in the mixture (Fig. 7).

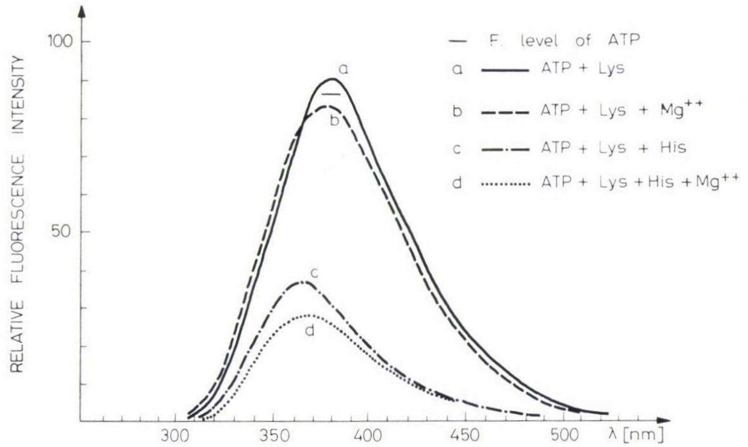


Fig. 7. Comparison of the ATP fluorescence intensity spectra for the effect of Lys and His in the absence and presence of Mg^{++}. Excitation at 300 nm

The reducing effect of Mg on the fluorescence intensity occurs through chelate formation, while the effect of Lys is manifested by the reduction of the fluorescence-quenching effect of His. This is supported by Fig. 3(b), showing that the fluorescence of ATP is reduced to the greatest extent by His. Thus, Lys loosens this high fluorescence quenching both by simple interaction and by chelate formation. The loosening effect of Lys is supported by the additive character of the fluorescence spectrum of the ATP-Lys mixture.

Maximum excitation of TML is achieved at a wavelength of 350 nm, and its fluorescence maximum is exhibited at 405 nm. In spite of this, we recorded the fluorescence spectrum of TML when excited at 300 nm for the sake of comparison with the fluorescence spectrum and intensity of ATP, though excitation at this wavelength gives only 17% of the intensity obtained at 350 nm. The fluorescence spectra of two TML solutions of different concentrations (5 and 20 μmole/ml) are shown in Fig. 8.

The fluorescence of TML is slightly changed by Mg ions or His separately, the former causing a reduction from 100 to 98, and the latter from 100 to 96% in the fluorescence intensity of TML, while the three components together (TML, His, Mg) show a slight additivity, increasing the intensity from 100 to 102% (Fig. 9). This is in contrast to non-methylated lysine, where a strong interaction and chelate formation is produced with His or His + Mg, since a fluorescence quenching of about 50% can be observed (see Fig. 6). On the basis

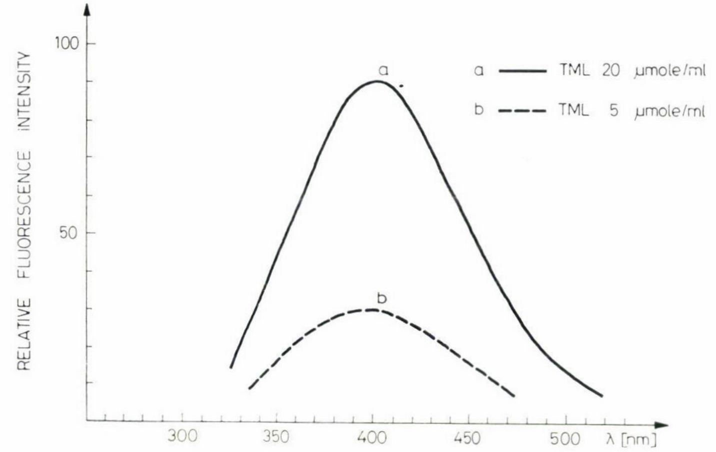


Fig. 8. **Fluorescence spectra of TML in two different concentrations. Excitation at 300 um**

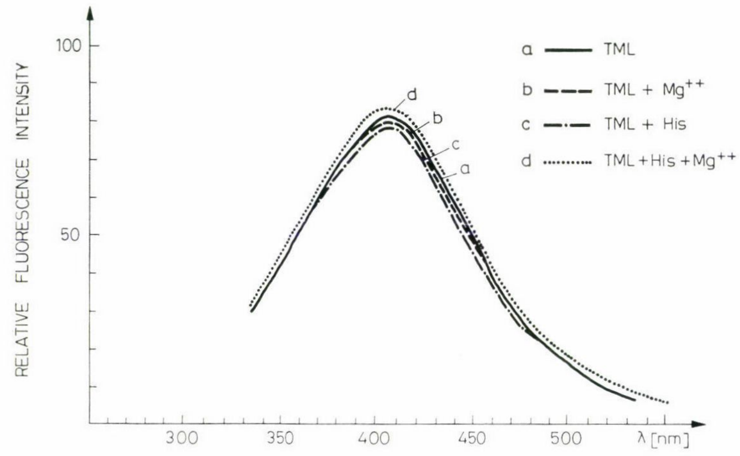


Fig. 9. Small variation in the fluorescence intensity of TML on the effect of Mg^{++} and His. Excitation at 300 nm

of this observation alone it was to be expected that TML would produce a quenching of ATP fluorescence, in contrast to lysine.

The effect of TML on the fluorescence intensity of ATP. TML reduces the fluorescence of ATP very strongly, from 100 to 18%. This quenching of fluorescence is a proof of the interaction between TML and ATP, even in the absence of Mg. This yields a simple proof of the weighted behaviour of TML towards ATP, and this is connected with the quaternary N-trimethyl group.

The presence of Mg produces a loosening effect, since thef luorescence-quenching effect is only from 100 to 24% in a reaction mixture containing TML, ATP and Mg. However, the presence of His further increases the fluorescence- quenching effect in the four-component TML, ATP, His, Mg mixture, but the loosening effect of the Mg ion can again be observed, as compared to the latter four-component mixture, since in the control mixture of TML, ATP and His, prepared with the omission of Mg, the fluorescence intensity decreases from 100 to 5%.

In myosin there are two Cys side-chains with different activities towards the substrate, the quickly reacting SH_1 and the slowly reacting, buried SH_2 chains, which are made responsible for the formation of the enzyme-substrate intermediate (REISLER *et al.* 1974). Therefore, the interaction of Cys with the substrate, and with those of the amino acids of myosin which presumably participate in the formation of the intermediate, have been investigated.

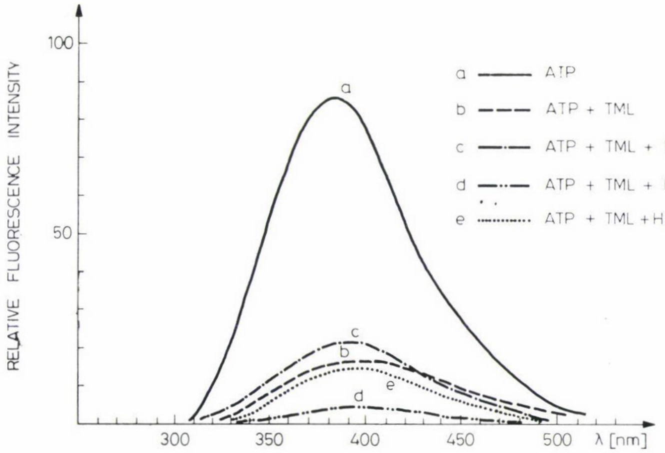


Fig. 10. Comparison of the fluorescence-quenching effect of TML in the absence and presence of Mg^{++} and His on the modification of fluorescence spectra of ATP. The concentration of components was 1.25 μmole in all cases. Excitation at 300 nm

A 5 μmole/ml Cys solution, investigated at an acidic pH and an excitation corresponding to that of ATP (300 nm), exhibits a fluorescence as low as one division, and at a pH of 8, as high as 6.5% of the ATP fluorescence. This intensity is reduced by Mg and His ions to 2.5% and 3.5%, respectively, even in the presence of Cys (Fig. 11). These data can be interpreted as indicating that in the presence of Mg ions, Cys is capable of both a simple interaction and chelate formation.

Cys causes a considerable quenching of ATP fluorescence, reducing its intensity from 100 to 4%. Fluorescence quenching is similar to that of the Cys, ATP, Mg and the Cys, ATP, His mixtures, both being reduced from 100 to 5%, although the simultaneous presence of all four components significantly reduces the fluorescence quenching, from 100 to 34%.

The fluorescence of phosphorylated histidines only slightly exceeds that of distilled water or its Raman scattering, even in instruments of high sensitivity. Although P-His markedly quenches ATP fluorescence from 100 to 4%, the presence of Ca ions and Mg ions moderates the fluorescence quenching from 100 to 63% and from 100 to 49%, respectively. Considerations lead to the conclusion that the strong interaction between P-His and ATP is of no small significance in the phosphorylation of histidine in myosin, but that there is a highly important effect in the myofibrils, where, in the absence of Ca ions, the interaction of ATP prevents the two kinds of filament from interacting.

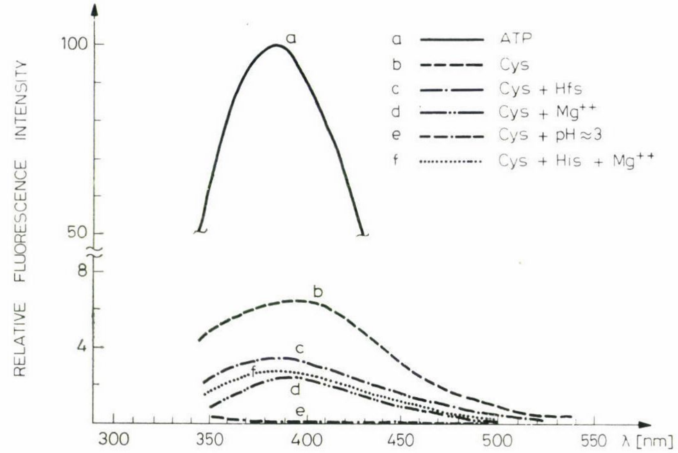


Fig. 11. Variation of the fluorescence intensity of Cys in the presence and absence of Mg^{++} and His at pH 8.0, and comparison of the fluorescence intensity of Cys and ATP at pH 3.8

Discussion

The object of our experiments was to clarify whether in vitro interaction or chelate formation in myosin activity between the contact amino acids had so far been proved, primarily between histidine and the substrate of myosin, ATP. The experimental results show that histidine interacts directly with ATP, as a consequence of which the fluorescence of ATP is strongly reduced, while in the presence of Mg ions this fluorescence quenching is moderated, owing to a change in structure.

Me-histidines give a more moderate interaction with ATP, the lowest fluorescence quenching being produced by 3-Me-His present alone in myosin (see Fig. 3); in the presence of Mg, the chelate of this amino acid is indicative of the loosest structure (Fig. 4).

Lysine does not interact directly with ATP. Even in the presence of Mg ions, the effect is scarcely perceivable (Fig. 7) and is presumably due purely to the Mg-ATP interaction (Fig. 1). At the same time, both interaction and chelate formation are observable between the amino acids Lys, His and the Mg ion (Fig. 6). In the ATP-His interaction and chelate formation Lys produces a loosening effect (Fig. 7). The situation is different when lysine is methylated.

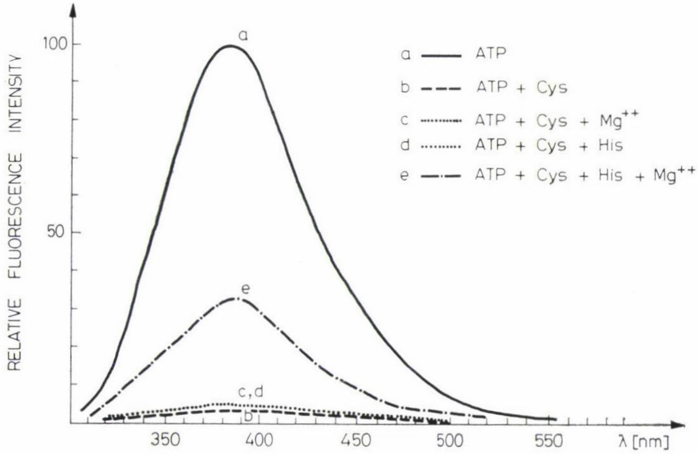


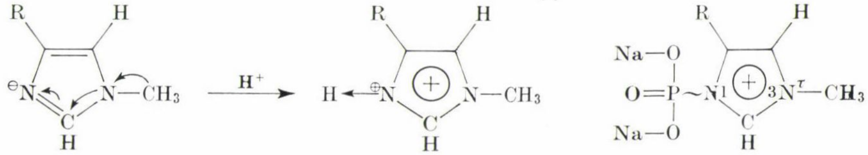
Fig. 12. Effect of Cys on fluorescence intensity of ATP spectra in the absence and presence of Mg^{++} and His at pH 3.8. Excitation at 300 nm

Quaternary trimethyl lysine does not interact with His even in the presence of Mg ions, but a very strong interaction develops with ATP, and this is further enhanced by the presence of His and Mg ions (Figs 9 and 10).

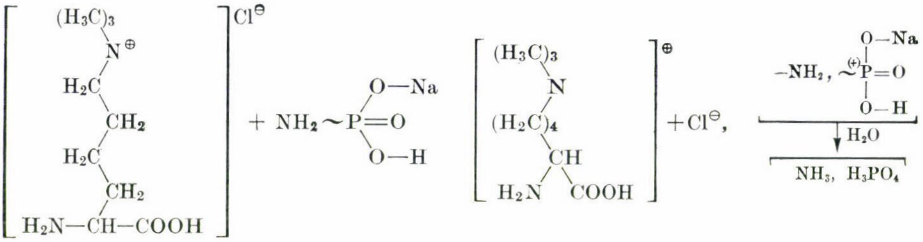
The interaction of Cys and His or chelate formation in the presence of Mg ions are difficult to evaluate by this method and no conclusions can be drawn (Fig. 11). However, a very strong interaction develops between Cys and ATP, where the most intensive fluorescence quenching can be observed (see Fig. 12). This interaction is moderated by the presence of His or Mg.

The amino acids investigated are the contact amino acids of myosin, which participate directly in the enzyme activity. Therefore, a knowledge of the sequence of these amino acids in myosin and phosphorylation seems very important.

Huszár—Elzinga (1971) isolated and reported the amino acid sequence of 3-Me-His peptide from subfragment-1. It is remarkable that 3-Me-His is



situated between two dicarboxyl amino acids, and this makes possible the presence of Me-His in the form of an imidazolinum cation in the peptide chain. Owing to the electron-repulsing effect of the —CH_3 group, the properties of 3-Me-His differ from those of histidine, because the electron-dense site of the N^{π}-atom of the imidazole ring is quickly protonized even under neutral conditions, and reacts easily with phosphoamidate.



The protonated form of 3-Me-His is very suitable for playing the role of the intermediate in the phosphorylation of myosin, and the 1-P, 3-Me-His obtained has a considerably higher stability than the simple N^{π}-P-His and is most stable in the peptide chain because there is no possibility for a rearrangement to 3-P-histidine. Mühlrad—Fábián (1968) found the presence of two lysines of importance in the enzymatic reaction of myosin. The ATP-ase activity of myosin stops after the TBS (trinitrobenzenesulphonic acid) reaction and TNP-Lys (trinitrophenyl-Lys) is formed. The TNP-Lys peptide has been isolated by Tokuyama *et al.* (1966), who found TNP-Lys peptides of two kinds:

Ser-TNP-Lys-Gly, Glu, Ser (Gly, Ala)
Asn-Pro-Pro-TNP-Lys.

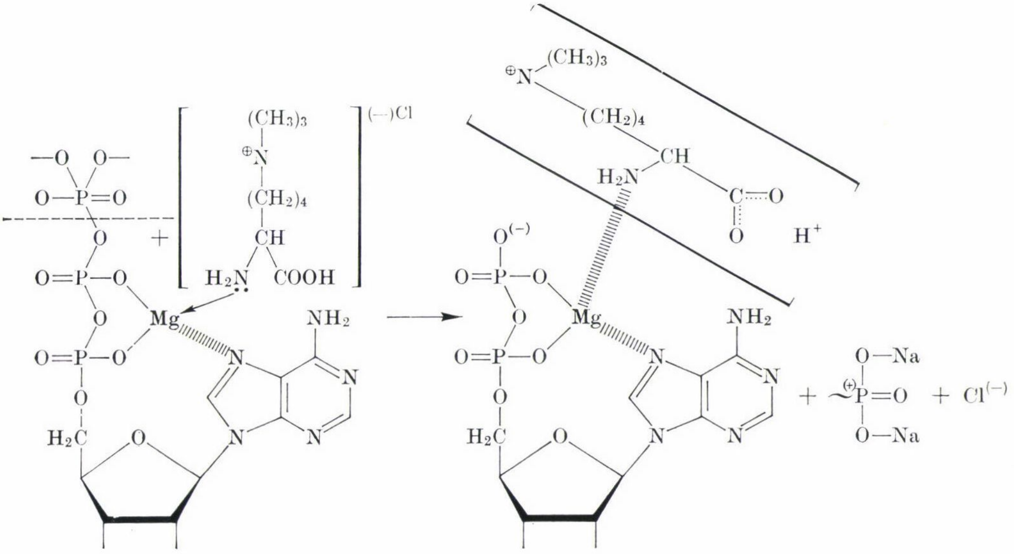
The occurrence of N^{ε}-P-Lys in myosin is unknown so far. On the basis of the interaction experiments, it is to be assumed that the Lys complex and the His complex do not participate in the formation of the same intermediate, but in that of different or successive intermediates.

Huszár (1972) isolated one monomethyl- and two TML-Lys peptides from myosin. The amino acid environment of the monomethyl-Lys peptide is not known, but the sequence of the two TML-peptides is as follows:

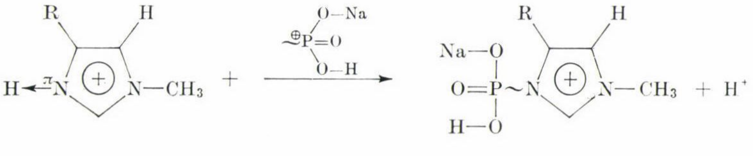
Ala-Thr-Asp-Thr-Ser-Asn-Phe-Me_3Lys-Lys-Lys
Tyr-Me_3Lys.

No trimethyl-N^{ε}-P-Lys can be obtained from TML by phosphoamidate, because free phosphoryl cation is formed, which ceases to exist in aqueous solution.

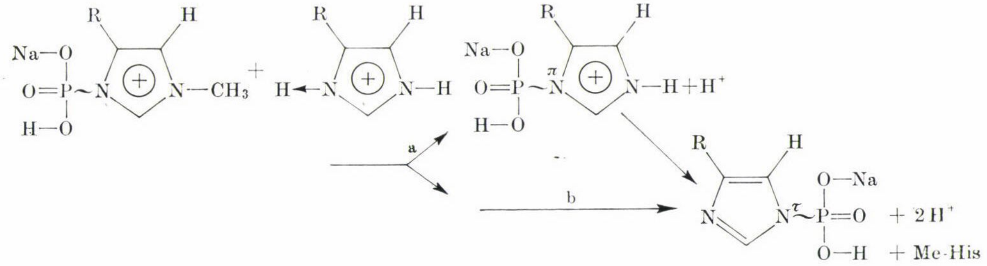
The formation of free phosphoryl cation raises the questions of whether the TML base may trigger the liberation of the phosphoryl cation, and whether it may be the initiator of a reaction series in the P-His synthesis process.



Under physiological conditions, the quaternary TML base of myosin liberates the phosphoryl cation from ATP, and this can participate in the phosphorylation of the histidine of the peptide chain. Our concept is demonstrated by the following reaction series:



It can be seen that the TML has an important role in the binding of ATP and in the liberation of the phosphoryl cation. The disturbance of the phosphorylation mechanism leads to the disappearance of the phosphoryl cation and inorganic phosphate appears, as in the phosphoamidate reaction, but the reaction has a different path up to 3-Me-His.



The proton production favours the formation of ATP chelates and increases the fluorescence intensity of the adenine (WATANABE *et al.* 1963). A change in the heavy chain fluorescenec intensity of myosin proportional to the nucleotide concentration was also observed by WERBER *et al.* (1972).

Myosin contains a quickly reacting (against NEM) SH_1 group, responsible for Mg^{2+} (K^+, EDTA)-ATP-ase activity, and an SH_2 group, responsible for Ca^{2+}-ATP-ase activity. The latter is also capable of binding ATP in the absence of metal ion (REISLER *et al.* 1974). Their reactivity is proved in our experiments by the strong Cys-ATP interaction and by Mg-chelate formation. The SH_1 and SH_2 peptides have been isolated by YAMASHITA *et al.* (1974). The function of the two SH peptides is an indication of the beginning of two different reactions. The further elucidation of their function is complicated by the fact that two SH_1 peptides of closely similar structure were isolated. The role of the Lys peptide is not clear, and neither is the mode of phosphorylation of histidines at different sites on myosin.

Acknowledgement

The authors are grateful to Prof. Antoni for his deep interest and valuable discussions throughout these investigations, and thank Mrs Bajna and Mrs Bökönyi for their skillful technical assistance.

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 25 (3—4), pp. 365—380 (1976)

LIME REQUIREMENT FOR TRITICALE IN RELATION TO OTHER SMALL GRAINS

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Four soils with pH values below 5.0 were limed at 0.0, 1.0 and 2.0 units of lime recommendations for wheat. For comparison, another soil with a pH value of 5.8 also received the equivalent of 0.0 and 7.3 mt/ha of lime. Three hexaploid triticale cultivars, one wheat and a rye were grown for six weeks on the limed soils. In a subsequent experiment, six different cultivars each of triticale, wheat, and barley, and two rye varieties were grown for eight weeks. Both experiments were conducted in growth chambers. The application of lime rates recommended for wheat in Alabama increased the growth if 6TA 131 and Trailblazer triticales on two of the soils, that of 6TA 385 on three soils, and that of Abruzzi on none of the soils, but the dry matter of Arthur wheat increased on all four soils for which lime was recommended. Liming the fifth soil which had an original pH of 5.8 did not increase plant growth. When averaged over the five soils, liming increased shoot dry weight by 87%, 51%, and 20%, respectively, for wheat, triticale and rye. In a high Al and Mn limed soil the average shoot growth of various barley cultivars was 2.0, that of wheat 1.4, that of triticale 1.2 and that of rye 1.0 times the growth in the unlimed soil. However, increasing the lime application to rates higher than those recommended for wheat did not generally increase plant growth. The average concentrations of Ca, Mg, Al, Mn and P in triticale were similar to those of wheat but less than those of rye. Tolerance for soil acidity was rye > wheat > barley.

Introduction

Triticale is a man-made crop produced by crossing wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.). Although the existence of triticale has been known for the past hundred years its potential as an agronomic crop has been considered seriously only in the past two decades. Triticale has the potential for producing high forage yields like rye and high grain yields like wheat (Sapra *et al.* 1973). However, before the potential contribution of this new crop to alleviating the world food supply can be properly evaluated, suitable cultivars and cultural methods have to be developed for different areas.

The objective of this study was to determine lime requirements for triticale grown on different Alabama soils which are representative of the major soil types in the southeast of the U.S. As a first approximation, the lime requirements for growing triticales may be expected to resemble those of existing small grains since the crop is a cross of wheat and rye. The existing small grains, however, differ in their tolerance to soil acidity. For example, wheat and barley cultivars have been shown to differ in their tolerances to soil acidity in many areas of the southeastern region of the U. S. (Foy *et al.* 1965a, Foy *et al.* 1965b, Jones—Thurman 1957, Long—Foy 1970). Foy and co-workers (Foy *et al.* 1965a) and Reid *et al.* (1969) have shown that Al toxicity is a major growth-limiting factor for many small grain cultivars introduced to this region from other geographical areas which are normally less acid. Low base saturation (Pierre 1931), and Mn toxicity (Foy *et al.* 1969) in the acid soils of the southeastern U.S. have also been associated with the poor performance of various crops. Low P availability has been shown to be limiting to other crops in some acid soils (Pearson—Adams 1967).

Literature is very scarce on the fertilizer and liming requirements for triticale. In one of the few published papers Slootmaker (1974) recently studied tolerance to soil acidity in wheat-related species, rye, and triticale, and suggested that the D-genome contributed to the tolerance to soil acidity in hexaploid wheats. He also found a high degree of tolerance in triticale cultivars as a result of the addition of the rye genome. In our study hexaploid triticales (6TA), wheat, barley, and rye were tested for tolerance to soil acidity and response to liming.

Materials and Methods

Experiment 1. Response of Triticale to Lime Applications to Five Soils in a Growth Chamber. Five soils from various areas of Alabama which are known to differ in lime requirements for existing crops were chosen for the study. The chemical properties, lime and fertilizer requirement of these soils as determined by the Auburn University Soil Testing Laboratory are shown in Table 1. Lime and fertilizer recommendations were based on wheat requirements.

Soils were screened to pass 5 mm sieve openings and lime was applied to bulklots at rates equivalent to 0.0, 1.0 and 2.0 units of lime recommendation on each soil. However, with Decatur silty clay loam for which no lime was recommended, 0.0 and 7.3 mt/ha of dolomite

Table 1

Soil test and lime and fertilizer recommendations on soils used for evaluating triticale response to liming

Soil	pH	Soil Tests				Recommendations**				
		P	K	Mg	Ca	Limestone	Mg	N	P_2O_5	K_2O
		kg/ha				mt/ha		kg/ha		
Decatur sic l	5.8	88	143	462	*	0.0	0	110	66	44
Dickson si l	4.9	33	99	143	*	7.7	calcite	110	132	0
Greenville s l	4.5	99	77	99	*	8.9	dolomite	110	66	66
Hartsells fs l	4.9	33	88	66	*	6.7	dolomite	110	132	66
Norfolk s l	4.7	209	66	44	*	3.3	dolomite	110	44	66

* See limestone used

** Lime and fertilizer recommendations were made by the Auburn University Soil Testing Laboratory.

were used. The latter rate was the average of lime recommendations on Dickson silt loam and Hartsells fine sandy loam which are found in the same geographical area as the Decatur soil. Lime was equilibrated with the soils in two cycles each consisting of wetting the soil to field capacity for two days and air drying the samples for four days. Fertilizers were applied at rates indicated in Table 1. Three kg of the treated soils were placed in tin cans lined inside with plastic bags. Hexaploid triticales, 6TA 131 and 6TA 385, were planted with all treatments replicated four times. Moisture content was adjusted to field capacity three times a week. The temperature in the growth chamber was kept at 22 ± 1 °C for 16 hours during the light period and 15 ± 1 °C in the dark until the plants were harvested after six weeks. Trailblazer triticale, Arthur wheat and Abruzzi rye were grown with the same soil treatments, lighting and temperature conditions in a different growth chamber. Shoots and roots were harvested separately at the end of six weeks.

Dry matter and Ca, Mg, Al and Mn concentrations in a 0.1 N HCl plant extract were determined. Exchangeable Ca, Mg, K and Mn extracted with 1 N NH_4OAc were measured by atomic absorption. Exchangeable Al extracted with 1N KCl was measured by the Eriochrome Cyanine R method (LONG—FOY 1970). Soil pH was determined in a 1 : 1 (w/w) soil and water mixture after 30 min. of stirring the mixture. Exchangeable K values (meq/100g) were Decatur 0.28, Dickson 0.07, Greenville 0.10, Hartsells 0.07 and Norfolk soil 0.09.

Experiment 2. Effect of Soil Acidity and Liming on the Growth of Triticale, Wheat, Barley and Rye in Hartsells Fine Sandy Loam. The soil was collected from the top 15—20 cm of a virgin Hartsells fine sandy loam *(Typic Hapludult)*. The soil contained an exchangeable (1 N KCl — extractable) Al level of 2.00 meq/100 g. Dolomitic limestone was applied to different soil samples at rates equivalent to 0.0, 3.4, 6.7, and 10.0 metric tons per hectare. These lime rates represented 0.0, 0.5, 1.0, and 1.5 units of lime recommended for wheat currently used in Alabama. Liming and fertilizer application were made as described in Experiment 1 for the same soil.

The wheat and barley cultivars used were selected from various states in the U.S. on the basis of their tolerances to Al as shown in Table 5. Ten plants were grown in each pot containing 3 kg soil with treatments replicated three times. The growth chamber lighting conditions were the same as described in Experiment 1 but the temperature was maintained at 27 °C for 16 hours with light and 21 °C for eight hours in the dark. After 8 weeks, shoots and roots were harvested separately. The roots were flushed with tap water and dipped for 30 seconds in a beaker containing 0.0001 N HCl and then successively in each of five beakers containing de-ionized water. Dry matter yields and Ca, Mg, Al, Mn, and P concentrations were determined.

Results

Triticale Wheat and Rye Responses to Liming Different Soils. Liming increased the exchangeable Ca and Mg and decreased the extractable Al and Mn, as expected (Table 2). The extractable Al practically disappeared from all soils when limed at the recommended rate, while relatively more exchangeable Mn persisted in Dickson and Greenville soils.

The forage growth of triticale 6TA 131 and 6TA 385 in different soils was significantly increased by liming Greenville sandy loam and Hartsells fine sandy loam (Table 3). The same was true of Dickson and Greenville for Trailblazer, but only of Greenville for Abruzzi rye (Table 4). A higher growth of triticale 6TA 385 was also obtained in limed Norfolk sandy loam. Wheat shoot dry matter significantly increased at recommended lime applications with the four soils. Furthermore, shoot dry matter in these four soils when limed increased by an average of 97% for wheat but by only 56 and 20%, respectively, for triticale and rye over the unlimed soils. Plant growth on Decatur silty clay loam, for which no lime was recommended, was generally greater than on any other soil and did not respond positively to lime applica-

Table 2

Soil pH and exchangeable cation at different lime rates applied to various soils

Soil	Lime applied*	pH	Exchangeable cations			
			Ca	Mg	Al	Mn
	mt/ha		meq/100g			
Decatur sic l	0.0	5.8	4.04	0.85	0.01	0.007
Decatur sic l	7.3	6.2	5.95	1.54	0.00	0.004
Dickson si l	0.0	4.7	1.98	0.47	1.41	0.053
Dickson si l	7.8	5.8	2.09	1.19	0.02	0.014
Dickson si l	15.6	6.7	2.11	1.91	0.01	0.007
Greenville s l	0.0	4.5	0.97	0.41	1.57	0.151
Greenville s l	8.9	6.7	2.25	1.33	0.02	0.065
Greenville s l	17.8	7.4	3.98	2.24	0.00	0.004
Harsells fs l	0.0	4.7	0.61	0.15	2.00	0.045
Hartsells fs l	6.7	5.7	2.92	1.03	0.00	0.006
Hartsells fs l	13.4	6.4	3.53	1.83	0.00	0.007
Norfolk s l	0.0	4.7	0.18	0.09	0.41	0.033
Norfolk s l	3.3	6.4	0.89	0.71	0.01	0.009
Norfolk s l	6.6	7.2	1.51	1.16	0.01	0.006

* Limes rates applied to each soil were 0, 1, and 2 units of lime recommendation. For Decatur sic l, lime applied was the average of amounts recommended for Dickson and Hartsells soils.

tion. The dry matter yields suggested that lime recommendation for wheat accurately predicted the amounts of lime needed to produce maximum triticale growth on all soils except for triticale 6TA 131 on Greenville, which needed more lime than is recommended for wheat.

Root growth only responded to lime on Greenville and Harstells with triticale, on Norfolk with wheat, and on Dickson and Greenville with rye (Table 4). The general lack of differences in root weight between plants grown in unlimed and limed soils was probably partially due to low root dry matter values and the inherent difficulty of incomplete root recovery from the potted soils. In the few treatments in which significant growth response to liming was obtained, root dry matter in limed soils averaged 190% or so more than in the unlimed soils, suggesting a large error in root recovery from the soils.

The Ca concentration in triticale 6TA 131 was significantly decreased by the addition of dolomite to Norfolk sandy loam, in contrast to the exchangeable Ca values which increased with the lime rate. No significant change in the Ca content of this cultivar was observed when the other soils were limed. Triticale 6TA 385 contained less Ca than 6TA 131 and the Ca concentration in 6TA 385 was increased by lime application, except on Norfolk soil where the Ca content in the plants decreased. Liming generally increased the Mg content in both triticales. Aluminium levels in plants were not affected by liming except in 6TA 385 plants grown in Greenville soil where the Al level at twice the recommended lime rates was lower than in the unlimed soil. The concentration of Mn in triticale was decreased by liming at the recommended rate, but no further decreases in Mn levels were caused by doubling this lime rate.

Although there was no relationship between the triticale growth responses to lime and the Al content in the plants, the highest dry matter increases in limed soils were obtained with Dickson, Greenville and Hartsells, which also contained the highest amounts of exchangeable Al (Tables 2 and 3). In addition, the lowest dry matter yields were obtained on these two soils and on Norfolk when no lime was added. These data suggest that the Al in the soil was a limiting factor for triticale growth but that the Al content in the plants was a poor indicator of the available Al in the soils.

Triticale Wheat, Rye and Barley Responses to Liming Harstells Soil. Application of dolomite to Harstells increased shoot growth except for rye cultivars which did not respond to lime (Table 6). The effectiveness of increasing lime rates became less apparent as the dolomite applied was raised from 6.7 to 10.0 mt/ha. However, when compared to the recommended 6.7 mt/ha, the highest lime rate decreased the shoot growth of 6TA 131, Kan. 989, and Abruzzi rye. Only Atlas 66 and Redcoat wheat and Colonial 2 barley required more lime than the recommendation for wheat. Fasgro 518 and Kan. 989 were more poorly adapted to the acid soil than the other triticales, but these two lines did not respond to lime either. Except for the low dry matter in Al-sensitive Arthur wheat and Kearney barley, there were no apparent differences in the growth of wheat and barley cultivars according to their differential tolerances to soil Al. In the unlimed soil (pH 4.8) the triticales 6TA 131, 6TA 203, and Trailblazer grew at least as well as rye and better than all the other cultivars. These triticales and also 6TA 385 have been shown to perform better than other triticale lines when tested at different locations in Alabama (unpublished data). Thus, among the triticales which appear to be well-suited to the acid soils of Alabama, according to ongoing field trials, only 6TA 385 did not perform as well as rye on the unlimed Hartsells soil. The average growth increase in the limed soil was triticale 19%, wheat 36%, barley 97% and rye no increase, when compared with growth in the unlimed soil.

Liming significantly increased the root dry weights of the triticales 6TA 203 and 6TA 385, five of the six wheat cultivars, Kearney barley, and rye (Table 7). The root dry weights of Kan. 989 and Trailblazer triticales were reduced by liming the soil. Applications of 10.0 mt/ha of dolomite were excessive in terms of root growth in some of the cultivars compared with the root

Table 3

Dry matter and mineral composition of triticale

Soil	Lime applied mt/ha	6TA 131				
		Dry matter yield* g/8 plants	Ca %	Mg %	Al ppm	Mn ppm
1	6.0	3.12^{+}	0.65^{+}	0.24^{+}	302^{+}	196^{+}
1	7.3	2.21^{+}	0.78^{+}	0.43^{+}	332^{+}	272^{+}
2	0.0	2.43ab	1.08ab	0.38c	308ab	1113b
2	7.8	2.54ab	0.77abcd	0.68b	284ab	135d
2	15.5	2.38ab	0.86bc	0.83ab	271b	285cd
3	0.0	1.17cd	0.62cd	0.28cd	265a	2068a
3	8.9	1.98b	0.61cde	0.47c	286ab	179cd
3	17.7	3.01a	0.41de	0.73b	275b	141d
4	0.0	0.96d	0.77abcd	0.19d	314ab	727bc
4	6.7	1.94b	0.60cde	0.67b	306ab	106d
4	13.3	2.30ab	0.96ab	0.80ab	305ab	32d
5	0.0	1.84bc	0.68bcd	0.25cd	337a	1914a
5	3.3	2.54ab	0.19f	0.37c	307ab	229cd
5	6.7	2.43ab	0.27e	0.97a	307ab	125d

1. Decatur sic l 2. Dickson si l 3. Greenville si l

* Within each column any two values having a letter in common are not significantly different at the 5% level by the Duncan's Multiple Range Test. + Indicates average of 4 replications for which no statistical analysis was done.

growth at the recommended lime rate for many of the cultivars used; generally this lime rate did not cause significant changes in root dry weight when compared with 6.7 mt/ha.

The plant Ca concentration of triticales (0.68%) grown in unlimed soil was comparable to that of wheat (0.65%) and barley (0.74%) but less than the Ca content in rye (0.95%) (Table 7). Liming significantly increased the Ca concentration in shoots of the triticales 6TA 203, 6TA 385 and Fasgro 518 and in all the wheat cultivars, but decreased the Ca levels in barley (except for an increase with Kearney) and rye. Shoot Ca remained uniform at different lime rates with triticale 6TA 131, as in the first experiment, and with Kan. 989 and Trailblazer. Root Ca concentrations generally tended to increase with the increasing lime rate. Triticale 131 and Tambar 401 barley accumulated more Ca in the roots than other cultivars.

On the average, the Mg composition in triticale plant shoots (0.28%) in unlimed soil was also similar to that of wheat (0.20%), but lower than the Mg *at different lime rates applied to various soils*

Soil	Lime applied mt/ha	6TA 385				
		Dry matter yield* g/8 plants	Ca %	Mg %	Al ppm	Mn ppm
1	6.0	2.28^{+}	0.52^{+}	0.20^{+}	189^{+}	198^{+}
1	7.3	2.42^{+}	0.50^{+}	0.34^{+}	186^{+}	171^{+}
2	0.0	2.13bc	0.45bc	0.20de	175bcde	316c
2	7.8	2.42ab	0.65a	0.68b	193bc	107de
2	15.5	1.82c	0.54abc	0.73b	205ab	97e
3	0.0	1.55c	0.25e	0.10e	252a	1463a
3	8.9	2.52ab	0.59abc	0.61bc	202ac	125de
3	17.7	1.87c	0.25e	0.93a	150bcde	174d
4	0.0	0.73d	0.23e	0.12e	145cde	312c
4	6.7	2.11bc	0.43cd	0.47c	123de	80e
4	13.3	2.41b	0.59ab	0.64bc	146cde	118e
5	0.0	1.50c	0.45bc	0.12e	135dc	1099b
5	3.3	2.90a	0.16e	0.34cd	151cde	98de
5	6.7	2.39b	0.23e	0.25de	142cde	79e

4. Hartsells fs 5. Norfolk l s.

levels in barley (0.33%) and rye (0.40%) (Table 8). Dolomite increased the Mg levels in the plants; the highest Mg concentrations in the shoots were obtained at 10.0 mt/ha for the four species of plants. Magnesium concentrations in the roots increased with the liming rate for triticale, wheat and barley, but the Mg levels decreased in rye grown in limed soils.

The concentration of Al in shoots was only significantly decreased by liming in 6TA 203, Arthur wheat, and McNair and Kearney barley (Table 9). There was otherwise no separation of wheat and barley cultivars according to their known differential tolerances for soil Al, suggesting that the Al in Hartsells soil was not the major growth-limiting factor. Trailblazer triticale accumulated less Al in the shoots than the other cultivars. The average shoot Al concentrations (ppm) were triticale 431, wheat 418, barley 440 and rye 453 ppm. The aluminium content in the roots was not related to soil pH.

The manganese concentration in the shoots of all the cultivars was significantly reduced by the application of 3.4 mt/ha but no further changes were obtained by increasing the lime rate (Table 10). Similar changes were observed

Table 4

Dry matter yields of triticale, wheat, and rye at different lime rates applied to various soils

Soil	Lime mt'ha	pH	Dry matter, g/pot^{+}					
			Trailblazer triticale		Arthur wheat		Abruzzi rye	
			Shoot	Roots	Shoot	Roots	Shoot	Roots
Decatur sic l	0.0	5.8^{+}	1.73^{+}	0.51^{+}	2.42^{+}	0.44^{+}	2.66^{+}	0.59^{+}
Decatur sic l	7.3	6.2^{+}	1.84^{+}	0.30^{+}	2.27^{+}	0.45^{+}	2.44^{+}	0.62^{+}
Dickson si l	0.0	4.6	1.10cde	0.30bc	0.69ef	0.31b	1.44d	0.31c
Dickson si l	7.8	5.8	1.65ab	0.33bc	1.36bcd	0.32b	1.96bc	0.51ab
Dickson si l	15.5	6.7	1.74a	0.35b	1.92a	0.27b	1.88cd	0.47bc
Greenville s l	0.0	4.7	0.81ef	0.17d	0.67ef	0.34b	1.75cd	0.31c
Greenville s l	8.9	6.7	1.14cd	0.34b	1.48abc	0.32b	1.97abc	0.47bc
Greenville s l	17.7	7.4	1.21bc	0.23bcd	1.87a	0.38b	2.42a	0.60ab
Hartsells fs l	0.0	4.7	0.60f	0.15d	0.54f	0.37b	0.57e	0.22d
Hartsells fs l	6.7	5.7	0.61f	0.21cd	1.04cde	0.23b	0.76e	0.28d
Hartsells fs l	13.3	6.4	0.85ef	0.29bc	0.93def	0.29b	0.72e	0.21d
Norfolk l s	0.0	4.8	1.50ab	0.52a	1.01de	0.37b	2.05abc	0.67a
Norfolk l s	3.3	6.4	1.54ab	0.53a	1.80ab	0.37b	2.02abc	0.54ab
Norfolk l s	6.7	7.2	1.44abc	0.56a	1.91a	0.73a	2.41ab	0.68a

* Within each column any two values having a letter in common are not significantly different at the 5% level by the Duncan's Multiple Range Test.

$+$ No statistical analyses were made for Decatur soil.

Table 5

Small grain cultivars used for evaluating lime effects in Hartsells fine sandy loam

Triticale	6TA 131, 6TA 203, 6TA 385, Kansas 989, Fasgro 518 and Trailblazer	
	Al-tolerant	*Al-sensitive*
Wheat	Thorne (Ohio)	Redcoat (Indiana)
	Atlas 66 (North Carolina)	Monon (Indiana)
	Seneca (Ohio)	Arthur (Indiana)
Barley	McNair (Alabama)	Kearney (Nebraska)
	Colonial 2 (North Carolina)	Cordova (Texas)
	Tambar 401 (Texas)	Hanover (Virginia)
Rye	Wrens Abruzzi and Vitagraze	

Table 6

Dry matter of triticale, wheat, rye, and barley at different lime rates applied to Hartsells soil

Cultivar	Lime, mt/ha							
	0.0	3.4	6.7	10.0	0.0	3.4	6.7	10.0
	Shoot				Roots			
	g/pot							
6TA 131 triticale	3.74	4.07	4.55	3.88	0.75	0.65	0.77	0.67
6TA 203 triticale	2.92	3.88	3.99	4.07	0.89	0.90	1.58	0.75
6TA 385 triticale	2.08	2.55	2.68	2.67	0.62	0.67	1.10	0.67
Fasgro 518 triticale	1.57	2.08	2.61	0.55	0.55	0.65	0.73	0.53
Kan. 989 triticale	1.74	1.60	1.45	1.00	0.76	0.56	0.44	0.38
Trailblazer triticale	4.19	4.49	4.49	4.38	0.65	0.36	0.33	0.47
Thorne wheat	2.34	3.05	2.97	3.21	1.21	1.58	1.86	0.90
Seneca wheat	2.32	2.66	3.24	3.15	1.05	1.58	1.48	1.10
Atlas 66 wheat	2.02	2.10	2.24	2.90	0.78	1.06	1.19	0.67
Redcoat wheat	1.99	2.74	3.04	3.55	0.93	1.45	1.58	1.38
Arthur wheat	1.34	1.95	2.42	2.39	0.91	0.90	0.79	0.68
Monon wheat	2.54	2.95	2.85	3.10	0.88	1.22	1.35	1.81
McNair barley	1.91	3.30	2.75	2.84	0.57	0.70	0.72	0.57
Colonial 2 barley	1.46	2.49	2.69	3.14	0.43	0.39	0.36	0.45
Tambar 401 barley	1.98	3.53	4.10	4.22	0.78	0.44	0.58	0.54
Kearney barley	0.70	1.60	2.08	1.88	0.64	0.83	1.04	0.82
Cordova barley	2.37	3.14	3.38	3.39	0.48	0.39	0.50	0.46
Hanover barley	2.01	3.14	3.50	3.86	0.81	0.55	0.48	1.09
Abruzzi rye	2.98	2.97	3.07	2.55	0.65	0.66	1.27	0.52
Vistagraze rye	3.27	3.46	3.44	3.14	0.73	1.15	1.33	0.66
LSD (0.05)	0.40				0.29			
LSD (0.01)	0.56				0.41			

with the roots. The average Mn concentrations (ppm) in shoots on the unlimed soil were triticale 709, wheat 681, barley 923 and rye 898.

Lime applications increased the P level in the shoots of most of the cultivars, except for decreases in 6TA 203 and uniform levels in Kan. 989 and Vitagraze rye (Table 11). The average P concentrations in the shoots were 0.27, 0.31, 0.22 and 0.37%, respectively, for triticale, wheat, barley and rye.

Table 7

Calcium content in small grain seedlings at different lime rates applied to Hartsells fine sandy loam

Cultivar	Lime, mt/ha							
	0.0	3.4	6.7	10.0	0.0	3.4	6.7	10.0
	Shoot				Roots*			
	% Ca							
6TA 131 triticale	0.75	0.76	0.77	0.72	0.43	0.41	0.41	0.34
6TA 203 triticale	0.49	0.68	0.82	0.54	0.16	0.16	0.16	0.17
6TA 385 triticale	0.53	0.66	0.78	0.78	0.11	0.10	0.10	0.08
Fasgro 518 triticale	0.66	0.67	0.80	0.68	0.14	0.15	0.14	0.18
Kan. 989 triticale	0.57	0.61	0.64	0.63	0.16	0.21	0.27	0.25
Trailblazer triticale	0.70	0.72	0.71	0.53	0.14	0.12	0.12	0.11
Thorne wheat	0.56	0.60	0.68	0.84	0.09	0.12	0.18	0.19
Seneca wheat	0.50	0.65	0.78	0.79	0.09	0.10	0.10	0.14
Atlas 66 wheat	0.60	0.70	0.83	0.76	0.10	0.16	0.22	0.22
Redcoat wheat	0.62	0.69	0.76	0.79	0.11	0.11	0.12	0.20
Arthur wheat	0.47	0.58	0.68	0.72	0.10	0.12	0.16	0.20
Monon wheat	0.37	0.48	0.62	0.64	0.07	0.09	0.11	0.04
McNair barley	0.75	0.67	0.59	0.60	0.06	0.09	0.12	0.14
Colonial 2 barley	0.79	0.67	0.44	0.56	0.10	0.13	0.18	0.06
Tambar 401 barley	1.20	0.77	0.65	0.60	1.10	0.11	0.12	0.14
Kearney barley	0.64	0.78	1.06	0.93	0.08	0.08	0.08	0.09
Cordova barley	1.01	0.72	0.79	0.70	0.05	0.09	0.18	0.12
Hanover barley	0.93	0.69	0.71	0.62	0.08	0.09	0.09	0.13
Abruzzi rye	1.07	0.92	0.92	0.92	0.37	0.38	0.37	0.20
Vitagraze rye	1.07	0.94	0.93	0.86	0.15	0.18	0.19	0.30
LSD (0.05)	0.13							
LSD (0.01)	0.17							

* Average of two determinations of a sample composited from three replications.

Discussion

The highest growth responses of 6TA 131, 6TA 385 and Trailblazer triticales and Arthur wheat to lime in various soils appeared to be related to the neutralization of high exchangeable Al originally in the soil, as in the case of Greenville, Hartsells and Dickson soils. However, in the case of Norfolk sandy loam improved triticale and wheat growth appeared to be associated with increases in the exchangeable bases, Ca and Mg, with liming. The results tended to suggest that either high Al, and possibly Mn, or low base saturation

Table 8

Magnesium content in small grain seedlings at different lime rates applied to Hartsells fine sandy loam

Cultivar	Lime, mt/ha							
	0.0	3.4	6.7	10.0	0.0	3.4	6.7	10.0
	Shoot				Roots*			
	% Mg							
6TA 131 triticale	0.39	0.56	0.73	0.76	0.07	0.08	0.09	0.07
6TA 203 triticale	0.21	0.44	0.66	0.52	0.11	0.18	0.23	0.27
6TA 385 triticale	0.31	0.55	0.79	0.89	0.18	0.21	0.27	0.41
Fasgro 518 triticale	0.23	0.46	0.68	0.52	0.22	0.25	0.28	0.40
Kan. 989 triticale	0.23	0.50	0.76	0.88	0.14	0.42	0.65	0.90
Trailblazer triticale	0.31	0.47	0.63	0.57	0.04	0.05	0.07	0.09
Thorne wheat	0.21	0.51	0.70	0.83	0.15	0.20	0.33	0.39
Seneca wheat	0.20	0.43	0.66	0.75	0.14	0.18	0.24	0.39
Atlas 66 wheat	0.24	0.46	0.68	0.74	0.24	0.21	0.22	0.35
Redcoat wheat	0.17	0.42	0.64	0.74	0.09	0.13	0.18	0.35
Arthur wheat	0.19	0.47	0.72	0.75	0.08	0.18	0.35	0.39
Monon wheat	0.19	0.40	0.57	0.69	0.07	0.13	0.20	0.14
McNair barley	0.28	0.53	0.56	0.65	0.15	0.27	0.37	—
Colonial 2 barley	0.41	0.52	0.70	0.77	0.03	0.05	0.08	0.05
Tambar 401 barley	0.39	0.58	0.68	0.70	0.09	0.08	0.07	0.05
Kearney barley	0.24	0.57	0.94	1.00	0.15	0.25	0.37	—
Cordova barley	0.34	0.54	0.74	0.71	0.02	0.05	0.06	0.04
Hanover barley	0.37	0.57	0.76	0.75	0.02	0.03	0.03	0.05
Abruzzi rye	0.41	0.60	0.76	0.85	0.85	0.70	0.64	0.49
Vitagraze	0.39	0.56	0.72	0.80	0.23	0.30	0.36	0.36
LSD (0.05)	0.11							
LSD (0.01)	0.15							

* Average of two determinations of a sample composited from three replications.

limited the triticale and wheat growth in different soils, with two exceptions: the triticales 6TA 131 and 6TA 385 did not respond to liming Dickson silt loam, which contained high exchangeable Al and Mn, 1.41 and 0.053 m.e./100g, respectively. The other exception was that Trailblazer growth did not respond to dolomite applied to Hartsells soil, which has an exchangeable Al value of 2.00 m.e./100g, and to Norfolk soil, with low available Ca and Mg. Using the lime recommendations for wheat in various soils produced maximum growth, suggesting that triticales require less or as much liming as wheat. Rye was not responsive to these lime rates on any of the soils. Lime rates higher than the recommended amounts generally either decreased or did not affect the dry

Table 9

Aluminium content in small grain seedlings at different lime rates applied to Hartsells fine sandy loam

Cultivar	Lime, mt/ha							
	0.0	3.4	6.7	10.0	0.0	3.4	6.7	10.0
	Shoot				Roots			
	ppm Al							
6TA 131 triticale	298	207	182	238	2.686	3.320	1.490	1.302
6TA 203 triticale	471	341	322	358	3.760	4.712	7.781	2.926
6TA 385 triticale	648	501	502	350	2.439	1.357	2.963	—
Fasgro 518 triticale	533	367	668	409	2.899	2.630	3.949	—
Kan. 989 triticale	653	684	815	653	2.368	3.750	2.119	2.893
Trailblazer triticale	124	189	224	189	2.106	2.098	2.291	1.176
Thorne wheat	286	454	342	307	1.489	2.406	4.082	1.931
Seneca wheat	385	454	326	272	3.849	950	1.522	1.502
Atlas 66 wheat	460	484	449	344	2.896	3.529	4.134	2.186
Redcoat* wheat	531	383	345	291	1.320	1.577	1.195	1.808
Arthur* wheat	781	568	466	453	3.282	2.265	8.288	2.411
Monon* wheat	442	448	420	349	3.364	3.064	2.925	2.987
McNair barley	707	387	367	292	3.183	3.150	—	2.792
Colonial 2 barley	606	423	293	319	1.209	1.549	1.066	1.563
Tambar 401 barley	385	185	131	195	1.059	1.538	1.676	1.087
Kearney* barley	1.764	1.310	711	653	2.859	1.446	—	1.750
Cordova* barley	309	203	220	223	1.281	1.486	1.398	1.228
Hanover* barley	330	208	148	192	1.317	2.535	1.508	909
Abruzzi rye	312	474	302	380	1.861	7.557	1.250	1.386
Vitagraze rye	282	283	336	346	4.464	1.069	983	1.608
LSD (0.05)	190							
LSD (0.01)	250							

* Average of two determinations of a sample composited from three replications.

matter obtained, depending on the soil type. Increases in Mg and reductions in Mn contents in triticale plants were more closely associated with the soil pH when the soils were limed than were the Ca and Al contents. This would suggest that triticale roots discriminate more against the absorption and/or translocation of Ca and Al than of Mg and Mn.

The average shoot growth response to lime on these soils indicated that Trailblazer triticale and Abruzzi rye had lower lime requirements than the triticales 6TA 131 and 6TA 385 and Arthur wheat. Wheat growth doubled in the four soils which needed liming, as a result of increases in the calcite or

Table 10

Manganese content in small grain seedlings at different lime rates applied to Hartsells fine sandy loam

Cultivar	Lime, mt/ha							
	0.0	3.4	6.7	10.0	0.0	3.4	6.7	10.0
	Shoot				Roots*			
	ppm Mn							
6TA 131 triticale	1.065	193	155	131	695	170	92	84
6TA 203 triticale	473	112	169	71	393	184	—	49
6TA 385 triticale	659	171	91	60	628	95	70	70
Fasgro 518 triticale	589	151	234	49	743	141	874	79
Kan. 989 triticale	634	116	57	64	649	241	114	99
Trailblazer triticale	834	160	109	93	668	209	70	34
Thorne wheat	655	142	83	71	485	97	80	57
Seneca wheat	686	185	93	84	447	116	39	40
Atlas 66 wheat	724	130	64	49	581	135	69	43
Redcoat wheat	742	207	94	83	366	102	52	23
Arthur wheat	656	185	111	75	328	120	125	37
Monon wheat	620	173	74	53	295	166	12	66
McNair barley	847	104	72	70	843	87	20	36
Colonial 2 barley	863	73	121	98	481	366	95	76
Tambar 401 barley	1.010	243	91	80	1.928	162	80	36
Kearney barley	1.090	279	112	106	636	89	80	26
Cordova barley	919	149	126	124	1.635	145	101	42
Hanover barley	810	146	117	112	1.272	266	97	63
Abruzzi rye	931	147	129	108	369	259	42	47
Vitagraze rye	865	151	119	130	676	56	36	41
LSD (0.05)		319						
LSD (0.01)		419						

* Average of two determinations of a sample composited from three replications.

dolomite applied. The average increase in dry matter in limed soils was 90% for triticale 6TA 385, 54% for 6TA 131 and only about 20% for Trailblazer and Abruzzi rye. The data tend to support SLOOTMAKER (1974) who suggested that the inclusion of rye genome in triticale makes it tolerant to high soil acidity. However, it is known that Arthur wheat, which was used in this experiment, is more sensitive to soil acidity (Al) than other wheat cultivars (FOY *et al.* 1974). Therefore, the greater response of this cultivar compared to that of 6TA 131 and Trailblazer triticales and Abruzzi rye to liming the four soils in the present experiment should not be interpreted to mean that wheats will always

Table 11

Effect of lime rates on phosphorus content in plants grown in the growth chamber

Cultivar	Lime, mt/ha							
	0.0	3.4	6.7	10.0	0.0	3.4	6.7	10.0
	Shoot				Roots*			
	% P							
6TA 131 triticale	0.14	0.16	0.23	0.20	0.21	0.16	0.14	0.09
6TA 203 triticale	0.52	0.26	0.25	0.23	0.23	0.20	0.18	0.06
6TA 385 triticale	0.25	0.24	0.32	0.31	0.19	0.18	0.17	0.28
Fasgro 518 triticale	0.25	0.26	0.35	0.32	0.10	0.10	0.08	0.14
Kan. 989 triticale	0.30	0.31	0.32	0.31	0.12	0.31	0.51	0.32
Trailblazer triticale	0.14	0.26	0.29	5.27	0.09	0.09	0.08	0.08
Thorne wheat	0.23	0.26	0.37	0.32	0.15	0.16	0.15	0.34
Seneca wheat	0.25	0.25	0.26	0.37	0.17	0.19	0.21	0.19
Atlas 66 wheat	0.26	0.27	0.26	0.35	0.18	0.21	0.22	0.21
Redcoat wheat	0.20	0.24	0.31	0.39	0.13	0.13	0.11	0.25
Arthur wheat	0.30	0.35	0.49	0.49	0.10	0.22	0.38	0.07
Monon wheat	0.30	0.32	0.29	0.42	0.11	0.10	0.15	0.05
McNair barley	0.32	0.33	0.34	0.42	0.18	0.15	0.12	0.12
Colonial 2 barley	0.10	0.09	0.08	0.23	0.09	0.10	0.10	0.06
Tambar 401 barley	0.10	0.17	0.26	0.20	0.07	0.08	0.11	0.08
Kearney barley	0.24	0.23	0.30	0.33	0.13	0.16	0.19	0.16
Cordova barley	0.11	0.17	0.25	0.28	0.06	0.06	0.08	0.06
Hanover barley	0.07	0.12	0.18	0.16	0.06	0.08	0.09	0.10
Abruzzi rye	0.30	0.34	0.48	0.40	0.15	0.28	0.44	0.22
Vitagraze rye	0.37	0.35	0.32	0.41	0.11	0.11	0.15	0.10
LSD (0.05)	0.09							
LSD (0.01)	0.12							

* Average of two determinations of a sample composited from three replications.

be more responsive to lime than triticale and rye on these soils. We may infer however, that 6TA 131 and Trailblazer triticales grow well in strongly acid Alabama soils and better than other lines of triticale (unpublished data) because they are more tolerant to soil acidity.

The high tolerance of Trailblazer to soil acidity was also found in Hartsells soil treated with four different rates of dolomite in the second experiment. The shoot dry matter of Trailblazer, Fasgro 518, and Kan. 989 was not affected by the different rates of lime application. The growth of 6TA 131, 6TA 203, and 6TA 385 increased to a maximum at 3.4 or 6.7 mt/ha of dolomite applied to Hartsells soil, which was also the case with the wheat cultivars. Therefore, triticales required either a similar or a slightly lower soil pH than wheat for maximum growth if they responded to liming, while other triticales grew well on the unlimed soil (pH 4.8). The triticales which have been shown to grow well in acid Alabama soils produced as much dry matter as rye but more than wheat on the unlimed soil. Compared with shoot dry matter on the unlimed soil, the triticales responding to lime showed about the same growth increase (25%) as Al-tolerant wheat cultivars (27%) and less than that of Al-tolerant barley (81%), and Al-sensitive wheat (45%) and barley (113%), but more than rye which did not respond to lime. The growth response increase of triticales which were more tolerant to soil acidity was intermediate (13%) between rye and Al-tolerant wheat cultivars. Therefore, on the average, triticales were nearly as tolerant to soil acidity as rye but more tolerant than wheat and barley.

The Ca, Mg, Al, Mn and P compositions suggested that the genetic control of nutrient uptake by triticale was inherited from wheat, while the growth suggested that tolerance to soil acidity was induced by a rye genome in triticale. The mineral composition in the two experiments indicated that Ca and Al tended to remain in the triticale roots, while Mg and Mn were translocated to the shoots in proportion to their availability in the soils. Both the growth and mineral analysis data indicated that Trailblazer was very tolerant to soil acidity. However, no direct comparisons could be made between the wheat and rye parents and the triticales tested since the origin or inheritance of the triticale entries is not exactly known.

Acknowledgements

This study was funded by the Cooperative State Research Service, United States Department of Agriculture (CSRS-USDA) Grant No. 316—15—22. The authors are indebted to Dr. S. M. Elgawhary for his assistance in statistical analysis.

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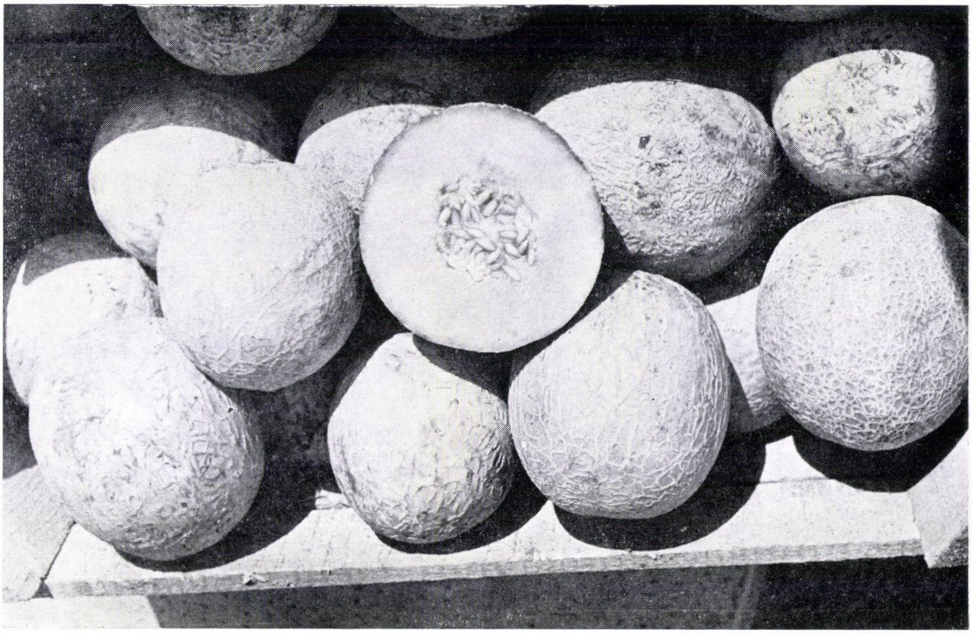
VARIA



"HYBRID 7" MUSK MELON

Taxonomic place: Cucumis melo L. ssp. *melo* MSF var. *reticulatus* NAUD.

Origin: Magyar Kincs × Queen Colorado, F_1 hybrid

State qualification: state certified variety, 1967.

Beginning of breeding: 1964, Budapest.

Breeder: Dr. Kálmán Mozsár, Budapest.

General characterization: the first Hungarian musk melon hybrid; a round variety with yellow flesh, usually succulent and tasty, resistant to disease, easy to transport and high yielding (TUZA 1968).

Morphological description:

Root system: medium deep.

Shoot system: medium strong, procumbent shoots with the surface completely covered by setae.

Stem: fleshy, greyish green, with medium long internodes.

Foliage: medium large, broad, spatulate leafblades with five shallow lobes; long petiole. The leaf is greyish green, of fine structure, covered by tiny hairs.

Flowers: with brimstone-coloured corolla.

Fruit: squat or somewhat oval, slightly ribbed, of light yellow colour, with a strong, thick network of cork on the surface. The average weight of the fruit is 0.80—1.20 kg. Rind is medium thick, hard (easy to transport!), flesh is orange-coloured, juicy, mellow, yet fairly firm, tasty, sweet. Refractometer value: 13.5%. Small fruit cavity.

Seed: cream-coloured; thousand-grain-weight: 23—26 g.

Biological character:

Development: medium growth vigour.

Vegetation period: from sprouting to the first picking 109—121 days are required (Tuza 1968). It ripens quickly, in large masses; 40—50 per cent of the yield can be harvested at the beginning of the ripening period (Molnár 1937). It ripens a few days earlier than the variety Magyar Kincs (Mándy 1971).

Resistance to disease: good (Molnár 1973).

Farm technology requirements:

Seeding: in hot bed 5—20th April, in sod or nutrition block (Tuza 1968).

Soil requirement: warm, freshly manured.

Productivity: with its 167—172 q/ha yield it is the highest yielding Hungarian variety, a rival to the variety Magyar Kincs, although at the beginning of ripening this produces a smaller number of fruit (Tuza 1968). A great advantage is the reliability of yield.

Region of cultivation: suitable for commercial production and household plots everywhere in Hungary (except for cooler soils).

*

Prepared at the Department of Botany, University of Agricultural Sciences, Debrecen.

Gy. Mándy

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STOMATAL RESISTANCE IN DIFFERENT PLANTS

The exchange of water vapour and carbon dioxide between the plant and its environment is a process of diffusion through the stomata and may be described by the following type of equation:

$$\text{flux} = \frac{\text{potential difference}}{\text{resistance}}$$

The "potential" of the soil–plant–atmosphere system is represented by the concentration of different properties (temperature, water vapour, carbon dioxide, etc.), while the "resistance"—to characterize it briefly—is connected with the "pathway" through which the property in question passes from one component to the other, e.g. water vapour from inside the plant (leaf) to the surrounding air (Evans 1963).

Methods for determining the transpiration of crops try to take into account the resistances influencing water transport in different ways (Penman–Schoflied 1951, Monteith 1965, Rijtema 1965, Milthorpe—Penman 1967). This raises, however, many problems because the rate of the water vapour and carbon dioxide exchange is considerably influenced by the age, development and water supply of the crop and by the external meteorological factors, since the stomatal opening depends on all these parameters (GAASTRA 1959, KUIPER 1961, KOZLOWSKI 1968, SZALAI 1968).

Stomatal reactions induced by the different factors can be expressed by the changes of resistance to vapour diffusion. Our task is to find out the relationships between environmental (meteorological and edaphonic) factors and diffusion resistance and, by means of these, to determine the crop resistance and to apply it to models for crop evapotranspiration.

The methods for calculating the crop resistance rely partly on meteorological elements — air temperature, wind speed, vapour pressure — measured on the crop surface and partly on the relation between leaf resistance and leaf area (SZEICZ—LONG 1970, SZEICZ—VAN BAVEL—TAKAMI 1973); because of this it is necessary to measure the stomatal resistance directly.

Investigations have been conducted in laboratories on a wide scale, to discover how stomatal resistance to vapour diffusion through the stomata depends on light and the water supply as the two most important factors (GAASTRA 1959, KARVÉ 1960, KUIPER 1961, MOSS 1963). There are few field measurements available, however, to give reliable values for the stomatal resistance in different phases of the plants during the growing season (MONTEITH *et al.* 1965, SZEICZ *et al.* 1973).

The construction of diffusion porometers made it possible to take field measurements and to obtain relationships between stomatal and crop resistance, or rather between resistances and the meteorological factors.

There are differences among the plant species and varieties in the number and position of the stomata; the number of stomata even changes in the same plant and leaf in the course of development and they differ in number on the upper and lower epidermis of the leaves. Therefore, to take measurements in the field, we need a light, portable apparatus, that can be used for leaves of any size. The LI-60 type porometer meets these requirements (KANEMASU *et al.* 1969), an improved version of the diffusion porometer constructed by Van Bavel and his co-workers in 1969.

As we have mentioned, the resistance to vapour transport is related to the stomatal opening and gives us some information on the amount of water vapour transported through the stomata in unit time and may inform us about the rate of stomatal transpiration.

In the summer of 1974 measurements were made on the leaves of potatoes, vines, paprika and maize with the LI-60 type porometer. In the figures all the hourly values of stomatal resistance are arithmetical means of ten measurements, or more precisely, they are means of measurements made on leaves in the same position on ten selected plants. The standard deviation was also determined, and in the figures the vertical dotted lines and numbers (σ) indicate its values. The temperature characteristic of the diffusion process was measured by means of a bead thermistor contained in the cup of the porometer and the values can be seen in some of the figures.

Potatoes. Fig. 1 shows the stomatal resistance measured on the sunlit upper epidermis of potato leaves; the plants were grown in a Mather-Thornthwaite type evapotranspirometer (4 $m^2 \times 1$ m) at the Agrometeorological Research Station, Keszthely. The plants were in the flowering phase. On 2nd July (a slightly cloudy day with a mean temperature of 19 °C), during the relatively short time of measuring, the stomatal resistance (r_S) increased from a value of 6 [sec/cm] at 9 a. m. to almost 20 [sec/cm] by 3 p.m.

The course of the resistance suggests that stomatal opening, which is a function of the turgor pressure in the leaves, changes even in plants kept in an evapotranspirometer. (In the 1 m deep tank, the water level was not particularly high. The ground water level was at a depth of 80—90 cm below the soil surface, so it is possible, that around midday the water supply of the plant could not keep pace with the rate of transpiration.)

It must be noted that we were unable to complete our measurements with observations important from a plant physiological point of view, such as a determination of the diameter

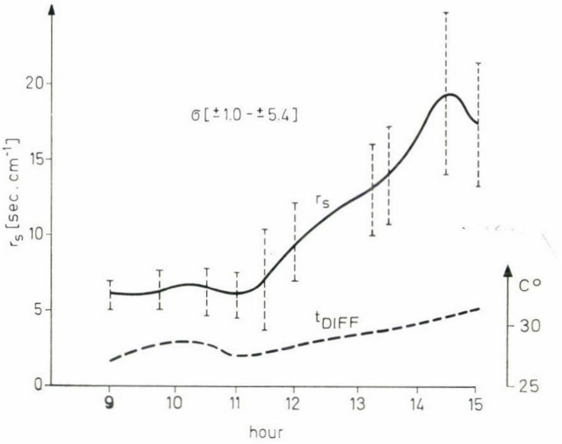

Fig. 1. Change of stomatal resistance (r_s) for potatoes (t_{diff} = diffusion temperature; σ = standard deviation), (Keszthely, 2nd July 1974)

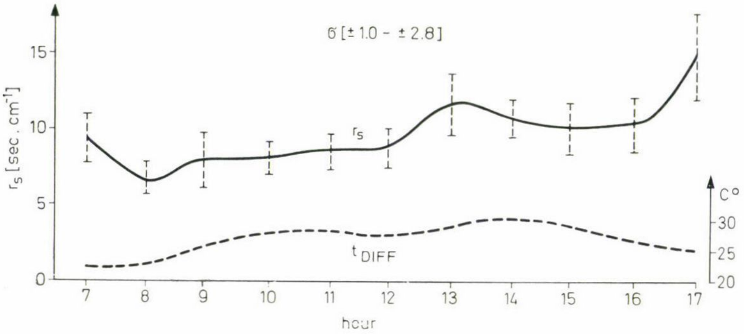

Fig. 2. Stomatal resistance for vine leaves (measured on the lower epidermis), (Abasár, 25—26th August 1974)

of the stomata, the water potential of the leaves, or the number of stomata per 1 mm^2 of the leaf. Failing these, we can only conclude from our general knowledge and on the basis of a comparison with other measured data, that about midday there is possibly a decrease in the stomatal diameter.

The values of standard deviation are fairly low; the maximum is about 30 per cent of the highest r_s value.

Between 25th and 31st August, 1974, we made measurements on vines, paprika and maize leaves.

Vine. The vine is a dicotyledonous plant; there are about ten times as many stomata in the lower epidermis of the leaf as in the upper one. Thus, it is easy to understand that at the end of August, by which time the available soil moisture had been mostly used for the yield, the stomata of the upper epidermis had an extremely high resistance; measurements could only be made with our instrument on the lower epidermis (Fig. 2).

During the day-time both the photo- and hydroactive reactions of stomatal movement are expressed; after 7 a.m. the value of the resistance decreases with increasing solar radiation, then early in the afternoon it seems to be slightly increasing. This is followed by a second minimum, then, after 4 p.m., r_s begins to increase again because of the decreasing radiation (at noon the value of radiation is 0.86 [gcal.cm^{-2}.min^{-1}], at 4 p.m. [0.28 gcal.cm^{-2}.min^{-1}]).

We calculated the coefficient of correlation between the stomatal resistance of vine leaves and the hourly values of air temperature, saturation deficit and wind speed, measured 1 m above the crop. The correlation proved to be significant at the levels of 1, 2 and 5 per cent or rather 99, 98 and 95 per cent.

Paprika. This also belongs to the class of dicotyledonous plants; so less stomata are to be found in the upper epidermis than in the lower one, although the difference is not so big as in the case of the vine. Thus, measurements could be made on both the upper and the lower epidermis of the paprika leaves (Fig. 3). The difference between the resistance of the two epidermis becomes significant after the maximum in radiation (1.36 [gcal.cm^{-2}.min^{-1}] at noon), when there is hardly any change in the values for the lower epidermis, while the values of r_s for the upper one becomes three times as high as it is before noon.

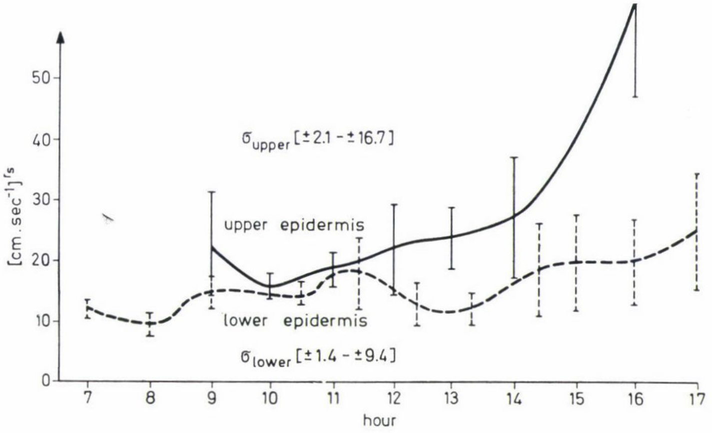

Fig. 3. Stomatal resistance on the lower (— — — — —) and upper epidermis of paprika leaves (Abasár, 29th August 1974)

Maize. Maize is a monocotyledonous plant. The difference in the number of stomata between the two epidemis is small; as an average, about 20 per cent more stomata can be found in the upper epidermis. It must be noted that because of the size of the leaves and other properties of the crop, measurements on maize leaves were the most difficult of all to carry out. The leaves bend, shade each other and thus there can be a considerable difference in the condition of the stomata even along the same leaf. Taking this into account we always took the measurements at two points, at one third and two thirds of the total length of the leaf.

The course of stomatal resistance for maize leaves can be seen in Fig. 4. The data in this figure are from measurements made on the second leaves of ten selected plants. Resistance has the lowest value at 9—10 a.m. then after 11 a.m. it rapidly increases and soon after 3 p.m. it became impossible to measure. This course of r_s indicates that after noon the water potential of the leaves may have considerably increased and it caused a rapid decrease in the opening of the pores. We were unable to observe the second minimum in stomatal resistance, often noticeable in the afternoon, on the two days we made the measurements.

Table 1 gives the values of solar radiation (G), air temperature (t), saturation deficit (Δe) and wind speed (u) at 1 m above the crop from the days selected for observations. In addition the table contains the values of crop resistance calculated by means of the following expression (Szeicz *et al.* 1973), knowing the leaf area-index (LAI):

$$r_c = \frac{r_s}{2\,\mathrm{LAI}} \cdot$$

Due to the lack of profile measurements above the crop we were unable to use any other method to calculate the crop resistance. The data of the table are of a purely informative nature.

Table 1

The values of the meteorological elements and the crop resistance
30th August 1974

hour	G (gcal · cm^{-2} · hour^{-1})	t (°C)	Δe (Hgmm)	u (msec^{-1})	$\frac{r_s}{2\mathrm{LAI}}$ (sec · cm^{-1})
8 a. m.	29.8	19.9	6.9	3.0	3.5
9 a. m.	44.6	21.4	8.3	3.2	2.2
10 a. m.	57.5	22.7	9.9	3.1	3.7
11 a. m.	64.8	23.5	10.8	3.2	3.5
12	67.1	24.5	11.7	2.7	7.6
1 p. m.	66.0	25.3	12.9	2.7	12.6
2 p. m.	61.9	26.1	14.4	2.0	15.0
3 p. m.	50.5	25.4	13.5	1.3	15.4

31st August 1974

hour	G (gcal · cm^{-2} · hour^{-1})	t (°C)	Δe (Hgmm)	u (msec^{-1})	$\frac{r_s}{2\mathrm{LAI}}$ (sec · cm^{-1})
8 a. m.	30,4	19.1	6.2	2.8	5.1
9 a. m.	45.7	21.0	8.0	2.8	3.6
10 a. m.	57.6	22.9	10.6	3.0	2.5
11 a. m.	64.8	24.2	12.2	2.7	2.5
12	66.7	25.0	13.5	2.3	6.9
1 p. m.	64.8	25.6	14.4	2.6	12.2
2 p. m.	57.3	26.3	15.3	2.1	16.0
3 p. m.	47.2	25.5	13.6	1.6	17.0

We will now deal with the results of measurements again made on maize leaves, this time on experimental plots at the Agrometeorological Research Station, Martonvásár, about 30 km from Budapest.

Fig. 5 shows the stomatal resistance for the leaves of a young plant (with 7—8 leaves). We took measurements at two points on each leaf, and the mean of them is given at the height corresponding to the level of the leaf. The resistance of the young leaves is fairly low, about 2—5 [sec/cm].

Profiles more interesting than the one given in Fig. 5 can be seen in Fig. 6, from data obtained on 2 days, 31st July and 1st August 1974. The maize crop was fully developed, in the flowering phase. On these two days the weather was quite warm, the daily mean temperatures being near 27 °C, and the saturation deficit about 14 Hgmm; there had not been any rain since July 22nd. Under these conditions we had to measure for such a long time (sec) with our instrument, that no corresponding values for the resistance could be obtained from the available calibration curves; so in the figure we give only sec-values (all calculated to 25 °C, so they are comparable).

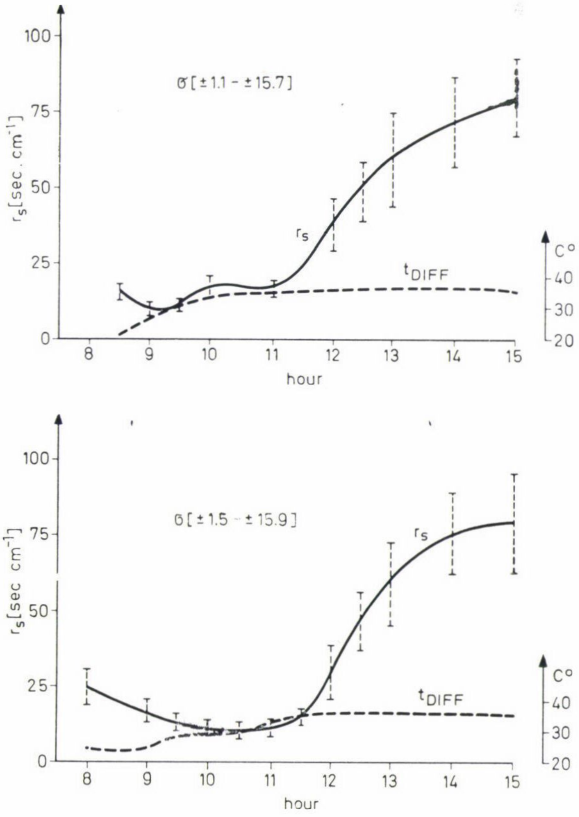

Fig. 4. Daily change of resistance for maize (measured on the 2nd leaves), (Abasár, 30—31th August, 1974)

According to the figure, the resistance — or more precisely the sec-values — increased from the morning till the evening, and at a given time they increased from the top to the lower leaves of the plant. Taking into account that the plant selected for the measurements was in the middle of the plot, the values reflect the effect of shading by the surrounding plants and the distribution of solar radiation within the crop. That is, the increase in the values downwards from the top of the plant is connected with the decrease of the amount of radiation with depth in the crop, while the higher ones in the afternoon are due to changes in the water content of the leaves.

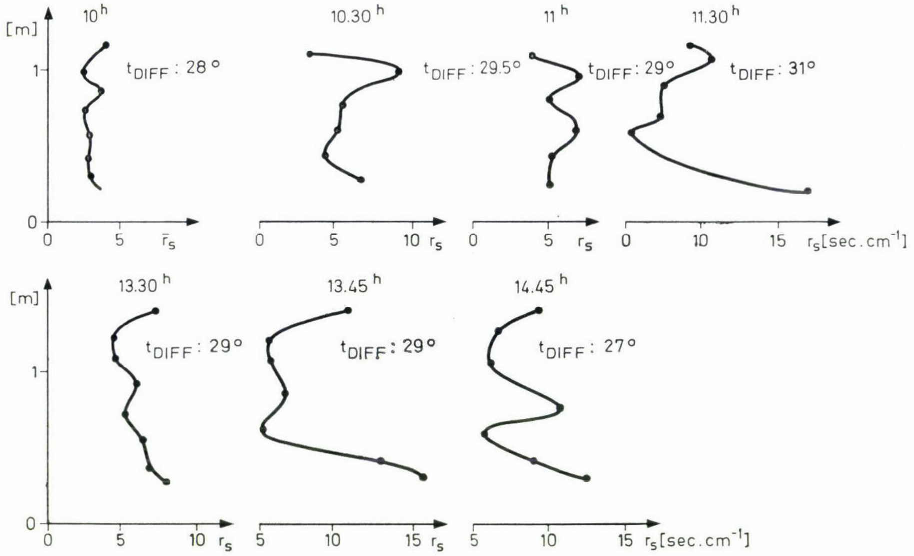

Fig. 5. **Profiles of stomatal resistance for a maize plant (on the X-axis r_s, on the Y-axis the height in meter), (Erdőhát, 9th July 1974)**

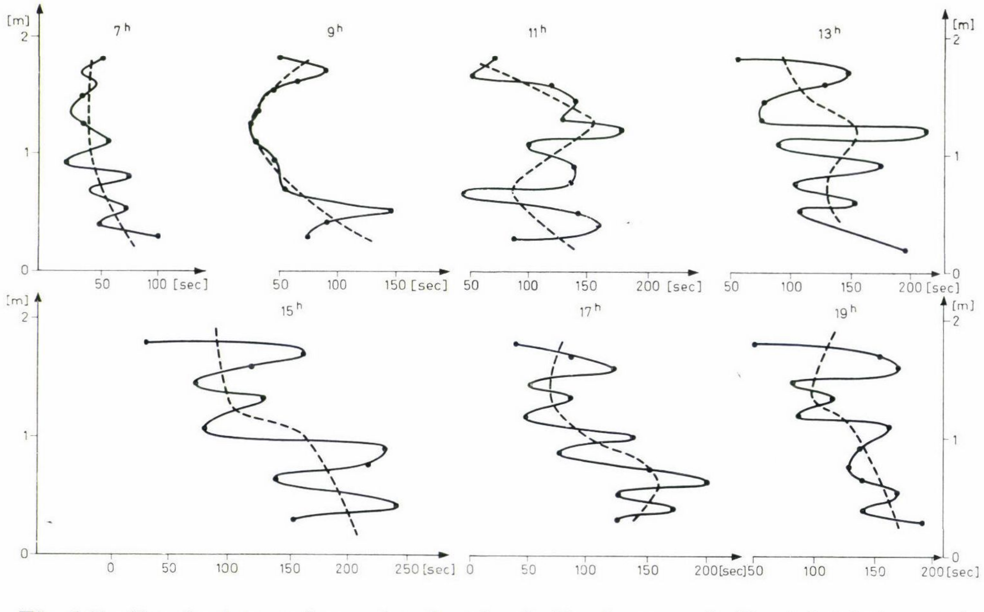

Fig. 6. Profiles of resistance for a maize plant (on the X-axis sec, on the Y-axis height in meter), (Erdőhát, 31st July—1st August 1974)

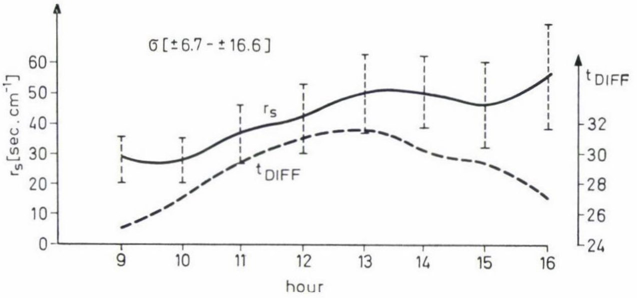

Fig. 7. The daily course of stomatal resistance for maize (for the 6th leaves), (Erdőhát, 11—13th September 1974)

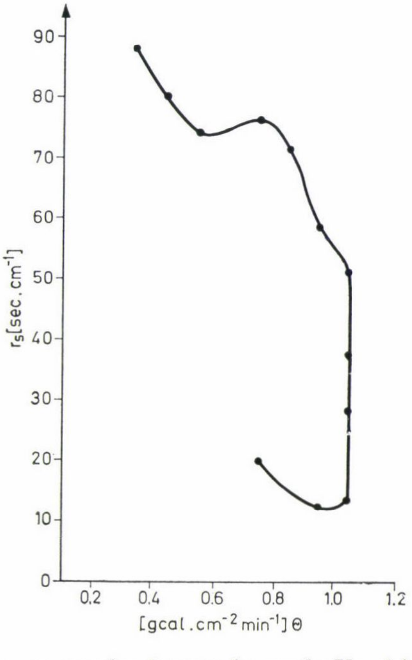

Fig. 8. The relation between stomatal resistance (r_S, on the Y-axis) and global radiation (on the X-axis) for maize (Abasár, 30—31th August 1974, days with clear sky)

Fig. 7 illustrates the stomatal resistance for maize leaves, using data obtained on 3 days in September. At this time the 6th leaves of ten plants, close to the ear, were chosen for the measurements. It could be seen that there was little change in the resistance in the leaves of a crop in the ripening phase.

The light and hydroactive reactions of stomata can be more thoroughly studied by means of the relationship defined between stomatal resistance and solar radiation, as well as between stomatal resistance and soil moisture. Our measurements are not sufficient to establish correct relationships, but as a first step we tried to characterize the relation between the values of stomatal resistance and solar radiation for maize by determining the correlation coefficient. We separated the data measured in the morning and in the afternoon, with the data measured in the morning and in the afternoon, with the following result: the data observed in the morning are characterized by a coefficient of 0.4, which, considering the number of cases, indicates a significant correlation at the 5%, or rather 95% level; for the afternoon data the correlation coefficient is 0.5, so the correlation is significant at a level of 1%, or rather 99%.

Fig. 8 shows the stomatal resistance as a function of radiation. The decrease in the resistance with the increase of light or rather radiation intensity — in other words in the morning — indicates the photoactive period of stomatal movement. The resistance decreases until the hourly value of solar radiation is about 0.8—1.0 [$\text{gcal.cm}^{-2}.\text{min}^{-1}$], then, while the radiation value remains constant, the higher values of resistance reflect the hydroactive reaction in the afternoon. On hot, sultry days the insufficient water supply causes a reduction in the water saturation of the cells and almost certainly results in a decrease in stomatal opening

The data we obtained using the diffusion porometer seem to be suitable for the characterization of the course of stomatal resistance, which is important from the point of view of water balance in plants. They give some information about coefficients concerning water loss by different plants under field conditions. To study the daily and seasonal variations of crop resistance and to get data good enough for use in evaporation models it is essential to complete our programme by collecting data which are sufficient from the plant physiological point of view, as well as by studying the heat and water balance of the crops.

*

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USE OF THE DYE BINDING METHOD (DBC) FOR ESTIMATING PROTEIN AND LYSING CONTENT IN RICE AND MAIZE

Breeding for high-protein and high-lysine cereal strains has been an active program in many countries in order to meet world protein demands. A rapid, reliable and cheap method is essential for the mass screening of protein content and quality in breeding work. The article describes the analytical conditions required for the application of the dye-binding method (Udy 1956, Udy 1972, Mossberg 1970, Kaul *et al.* 1970) in the chemical screening of rice and maize seeds.

The dye used in the experiment is Orange G (Feinchemie K-H. Kallies KG). Reagents were prepared as described by Mossberg (Mossberg 1970) for Acilane Orange G. 15.84 g citric acid and 2.98 g $Na_2HPO_4 \cdot 2H_2O$ were dissolved in distilled water. To this mixture, 0.30 g thymol in 3 ml ethanol and 2 g Orange G were added and the volume was made up with distilled water to 1000 ml. The pH of the solution was adjusted to 2.2. Its absorption spectrum has a maximum at 475 mμ. For estimating the dye-binding capacity of rice and maize, a 0.4 g finely ground sample was mixed with a suitable amount of dye solution in a 25 ml test tube. The mixture was then shaken on an universal shaker for 1 hour and filtered. The filtrate was diluted 20 fold with distilled water and the optical density measured at 475 mμ. The dye-binding capacity was calculated as mg Orange G bound to 1 g of dry sample. The protein content was calculated from the total nitrogen content of the sample as determined by the micro-kjeldahl method. The conversion factor was 6.25 for maize and 5.95 for rice. The basic amino acids were determined by paper electrophoresis. 100 mg samples of rice or maize were hydrolyzed in sealed tubes with 6 N HCl for 48 hours. The excess HCl was evaporated and the residue was dissolved in 5 ml 10 per cent isopropanol. The samples were run for 45 min. The solvent used was a mixture of piridine: acetic acid: water at a ratio of 2 : 4 : 994 (v/v/v). The lysine content of the maize was estimated colorimetrically according to Sisoev (Ermakob *et al.* 1972).

Table 1

Effect of dye/sample ratio on the dye-binding capacity of rice and maize

Dye/Sample ratio	DBC (mg'g)	
	rice	maize
8	—	11.2
10	14.6	13.4
13	—	14.9
20	15.19	17.2
30	16.2	—
40	16.2	20.0
80	16.2	20.1

A non-destructive version of the method consists of cutting the maize grains into two parts. Halves containing the embryo were stored in a dry place after covering the cut surface with a thin layer of paraffin to prevent infection by fungi. Other half seeds containing the endosperm were ground carefully in a mortar and used for the microdetermination of the dye-binding capacity. The procedure was similar to the macromethod: a 50 mg sample was mixed in a test tube with 2 ml of dye solution. After shaking for 1 hour and filtering, the mixture was diluted 30 fold and then measured at 475 mμ. The embryo-containing half seeds were germinated on moist filter paper and then sown in the field. The maize plants from these half seeds developed and formed ears normally.

As shown in Table 1, the dye-binding capacity of rice and maize greatly depends on the ratio of the dye solution (ml) and the amount of sample (g). Binding saturation is reached after

Table 2

Effect of shaking time on the dye-binding capacity of rice and maize

Shaking time (min.)	DBC (mg'g)	
	rice	maize
5	12.6	14.0
15	14.6	17.9
30	16.3	19.9
60	17.1	20.2
90	17.2	20.2
120	17.2	20.2

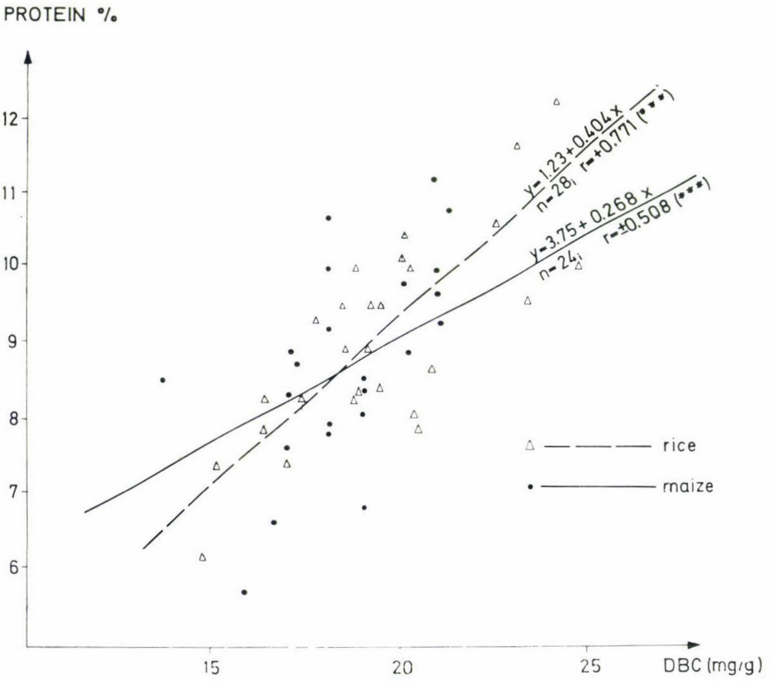

Fig. 1. Correlation between dye-binding capacity and protein content in rice and maize

shaking for 1 hour at the dye/sample ratio (v/w) of 20 and 40 for rice and maize, respectively.

Table 2 shows the effect of shaking time on the dye-binding capacity of rice and maize. Test tubes containing the sample and dye solution at an optimal ratio were shaken for different time intervals. The dye-binding capacity of both rice and maize reaches a plateau after shaking for 1 hour.

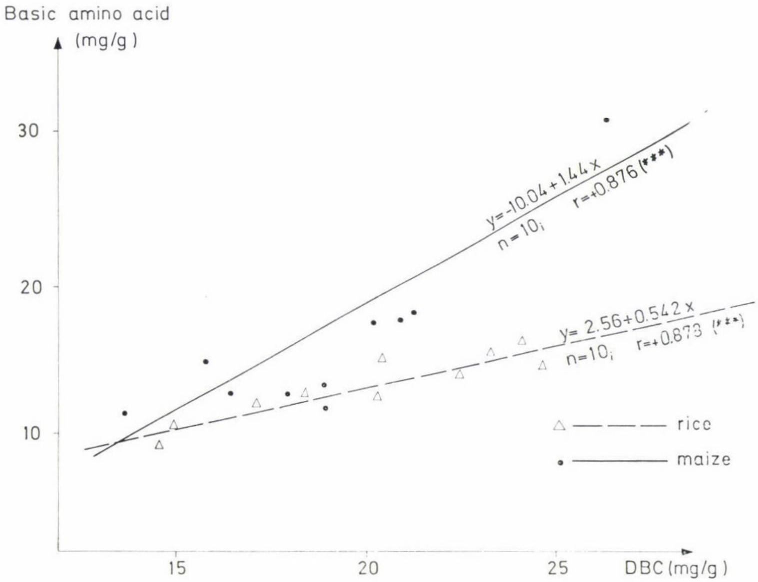

Fig. 2. Correlation between dye-binding capacity and basic amino acid content in rice and maize

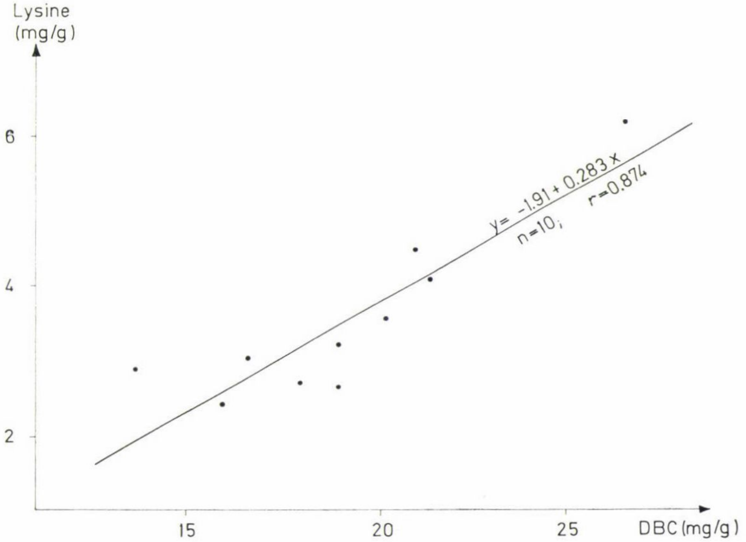

Fig. 3. Correlation between dye-binding capacity and lysine content in maize

Between 20° and 40 °C no effect of temperature on the dye-binding capacity of rice and maize was observed.

Fig. 1 shows the correlation between the dye-binding capacity and protein content of rice and maize. 28 varieties of rice with protein contents ranging form 6.24 per cent to 12.27 per cent and 23 varieties of maize with protein contents ranging from 5.76 per cent to 11.20 per cent were tested for dye-binding capacity. The correlation coefficient was found to be significantly high in rice, while in maize it was comparatively low. This can be seen clearly in the case of opaque-2 maize. Opaque-2 maize has the highest DBC value while its protein content is only at a medium level. Thus, in maize, there is a stronger correlation between DBC and protein quality than between DBC and protein content.

Fig. 2 shows the correlation between the DBC and basic amino acid content of rice and maize seeds. High correlation coefficients were obtained in both cases.

The DBC values were expected to correlate strongly with the lysine content. Fig. 3 shows such a correlation in maize. The correlation coefficient calculated was in fact fairly high ($r = +0{,}874^{+++}$). There was also a very high correlation between basic amino acid content and lysine content in maize ($r = +0{,}911^{+++}$).

High-lysine and high-protein strains of maize have been used extensively to improve the quality of maize protein (Mertz—Bates—Nelson 1964, Nelson—Mertz—Bates 1965, Baudet *et al.* 1968). High-protein rice strains have also been discovered. The selection of high-lysine maize strains is greatly facilitated by the opaque character of the seeds. Great efforts, however, have been made to select high-lysine maize strains with semi-opaque or normal appearance (Polimer 1973), as the negative effects of the o_2 gene on maize yield are difficult to overcome. The binding method is a very simple procedure suitable for the mass screening of the breeding materials used in improving the protein content and quality of rice and maize.

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INVESTIGATION OF THE EFFICIENCY OF FERTILIZATION ON AN EXTREMELY CALCAREOUS SANDY SOIL TESTED BY RYE

More than half of the relatively large sandy soil areas of Hungary consist of carbonate-containing soils, most of which are found in the Danube—Tisza Midregion. In this region there are some extremely calcareous soils in a number of places.

Major characteristics of these soils are: high $CaCO_3$ content, low humus content and poor nutrient level. The high $CaCO_3$ content is often found in fine distribution, which promotes the solubility and reactivity of the lime, decreasing thereby the fertility of the soil to a great extent. The extremely high lime content and its fine distribution in these soils essentially decrease the efficiency of nutrients (FULLER 1967, LÁNG 1962).

In the case of fertilization of the sandy soils of Hungary the amount of nitrogen fertilizer is a primary determinant (BAUER 1964, LÁNG 1972) and it is a precondition for the efficiency of phosphorus and potassium fertilization (ANTAL—EGERSZEGI—PENYIGEY 1966, LÁNG 1963). The efficiency of nitrogen fertilization is greatly increased by divided autumn—spring application (KOZÁK 1967).

The water regime of sandy soils is unfavourable; it decreases the availability of nutrients. Besides the problems mentioned, the question of the incorporation of the fertilizers arises, with a special regard to the fixation and mobility of the nutrients.

The effect of fertilization and the efficiency of nitrogen fertilizer combined with various rates of phosphorus and potassium fertilizers were studied in an extremely calcareous sandy soil of the Danube—Tisza Midregion.

Furthermore, the effects of divided application of nitrogen fertilizer, as well as that of different methods of incorporation of the fertilizers, were studied on the grain and straw yield of rye.

In Őrbottyán (former name: Őrszentmiklós) on a highly calcareous poor sandy soil area at the experimental station of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences a small-plot field trial was started in the autumn of 1968 with rye as the indicator plant, in a random block design with four replications, using the following treatments.

1. Untreated control
2. $P_{40}K_{50}$ and N_{75} distributed in spring
3. $P_{80}K_{100}$ and N_{150} ploughed in autumn
4. $P_{80}K_{100}$ and N_{150} disked in autumn
5. $P_{80}K_{100}N_{75}$ ploughed in autumn, and N_{75} applied in spring as top dressing
6. $P_{80}K_{100}N_{75}$ disked in autumn, and N_{75} applied in spring as top dressing.

Major agrochemical data concerning the ploughed layer of the experimental area: $CaCO_3$ content ranges between 10 and 15 per cent. The humus content is very low (0.4—0.6 per cent), organic matter is only found in the upper 30 cm layer. Of the nutrient content, total nitrogen is (according to Tyurin) 0.40—0.50 mg%, available AL-soluble phosphorus 5—7 mg%, potassium 3—4 mg%. Owing to the high $CaCO_3$ content the pH value is 7.5—8.0. In the mechanical composition of the soil the coarse sand fraction dominates, whereby the water conditions are highly unfavourable. According to the Hungarian genetic soil classification the soil is a slightly humous sandy soil.

Of the agrotechnical operations ploughing, disking and sowing were carried out mechanically, while the top dressing of the fertilizer in spring (March) was generally done by hand. Of the nitrogen fertilizers ammonium sulphate was applied in autumn and ammonium nitrate as top dressing in spring. In all three years of the trial the rye variety Kecskeméti H. was used as the indicator plant. The size of the plot was 45 m². Monthly amounts of precipitation in the different years of the trial are shown in Table 1, with deviations from the forty years' averages given for comparison.

Table 1

Amount of precipitation and deviation from the forty years averages
Őrbottyán – Őrszentmiklós

Month	1968/69		1969/70		1970/71	
	mm	deviation mm	mm	deviation mm	mm	deviation mm
October	4	–43	17	–30	10	–37
November	63	+18	74	+29	23	–22
December	47	+7	81	+41	81	+41
January	16	–11	61	+34	58	+31
February	122	+96	56	+30	16	–10
March	44	+9	47	+12	19	–16
April	11	–31	53	+11	35	–7
May	38	–24	24	–38	112	+50
June	118	+59	85	+26	64	+5
July	63	+15	120	+72	60	+12
August	42	–11	94	+41	38	–15
September	15	–31	13	–33	51	+5
Total	583	+53	725	+195	567	+37

Trends of grain yield and effects of fertilization in the different years and on the average of the three years are given in Table 2. In the untreated control the grain yield was 3.9 q/ha in the best year. This low level of yield is characteristic of the original fertility of the soils. The data of the trial were calculated by variance analysis (Sváb 1961).

In the first year of the trial significant grain yield surplus was only obtained with the divided application of $N_{150}P_{80}K_{100}$ kg/ha. When N_{150} was distributed on a single occasion in autumn there was no demonstrable difference in yield between the two methods of incorporation of the fertilizers (ploughing, disking). With the divided distribution of the above dose (N_{75}—N_{75}) the yield-increasing effect when the fertilizer was incorporated with a disk was significantly higher than when the fertilizer was ploughed under. Compared to the undivided autumn application of nitrogen, divided autumn – spring fertilization resulted in a significantly larger grain yield only when a disk was used for the incorporation of the fertilizer, while nitrogen ploughed under only tendentially increased the grain yield. Compared to a lower ($N_{75}P_{40}K_{50}$) rate of fertilization, only a higher ($P_{80}K_{100}$) base with divided autumn – spring (N_{75}—N_{75}) nitrogen fertilization incorporated by disk ensured a significant grain yield increase.

In the second year of the trial, due to more favourable precipitation conditions, the grain yield showed a more than 30 per cent increase. Compared to the control, all treatments — with the exception of treatment 4 — resulted in significant yield surpluses. If we take treatment $N_{75}P_{40}K_{50}$ as the basis for comparison, the higher rate — 150 kg/ha — of divided nitrogen

Table 2

Grain yields of rye

Treatment	Grain yield q/ha (86% dry matter)					
	1969			1970		
	q/ha	D	%	q/ha	D	%
1	1.9	—	100	3.9	—	100
2	4.6	2.7	242	9.2	5.3	235
3	4.8	2.9	253	8.1	4.2	207
4	5.0	3.1	263	6.2	2.3	159
5	6.2	4.3	326	13.5	9.6	345
6	9.8	7.9	516	14.2	10.3	364
LSD 5%		3.2			3.9	

Treatment	Grain yield q/ha (86% dry matter)					
	1971			Three years' average		
	q/ha	D	%	q/ha	D	%
1	2.1	—	100	2.6	—	100
2	7.6	5.5	362	7.1	4.5	273
3	5.1	3.0	243	6.0	3.4	230
4	6.9	4.8	328	6.0	3.4	230
5	11.3	9.2	538	10.3	7.7	396
6	14.3	12.2	681	12.7	10.1	488
LSD 5%		5.4			3.3	

fertilization and the application of $P_{80}K_{100}$ increased the grain yield significantly with both methods of incorporation. The divided autumn — spring (N_{75}—N_{75}) distribution of nitrogen was significantly more effective with both methods of incorporation than the single autumn application of the total N_{150} amount. Between the two methods of incorporation no mathematically demonstrable grain yield differences were found if the nitrogen was applied only in the autumn. Dividing the amount of nitrogen (N_{75}—N_{75}) for autumn and spring application, again no significant grain yield difference was found between the methods of incorporation, though the result of incorporation by disk was tendentially better than that of ploughing the fertilizer under.

In the third year of the trial the yield averages decreased compared to the previous year, yet they were essentially higher than those in the first year. With the exception of treatments where N_{150} was given in a single dose, all treatments resulted in significant surplus yields compared to the untreated control. Compared to the lower rate treatment, with $P_{40}K_{50}$ and N_{75} (applied in spring), demonstrable yield surpluses were only ensured when the fertilizer was incorporated by autumn disking, using the higher ($P_{80}K_{100}$) rate of treatment with nitrogen treatment applied on two occasions (75—75 kg/ha). When comparing divided (75—75 kg/ha) autumn — spring and single dose (150 kg/ha) autumn application of nitrogen, a significant

Table 3

Straw yields of rye

Treatment	Straw yield q/ha					
	1969			1970		
	q/ha	D	%	q/ha	D	%
1	2.4	—	100	11.7	—	100
2	5.4	3.0	225	20.2	8.5	172
3	6.3	3.9	262	24.0	12.3	205
4	7.2	4.8	300	19.7	8.0	168
5	8.7	6.3	362	33.0	21.3	282
6	12.0	9.6	500	33.2	21.5	284
LSD 5%		4.4			8.7	

Treatment	Straw yield q/ha					
	1971			Three years' average		
	q/ha	D	%	q/ha	D	%
1	3.4	—	100	5.8	—	100
2	10.3	6.9	302	12.0	6.2	206
3	6.9	3.5	202	12.4	6.6	213
4	8.8	5.4	258	11.9	6.1	205
5	15.0	11.6	441	18.9	13.1	325
6	18.5	15.1	544	21.2	15.4	365
LSD 5%		5.2			5.8	

grain yield difference can be found in favour of divided application. In the case of divided (N_{75}—N_{75}) autumn — spring application, incorporation by disking in autumn was significantly better than ploughing under in increasing the yield. In the case of nitrogen distributed in autumn in a single dose (N_{150}) only a tendency can be seen.

The yield date of the plots were averaged, then mathematically calculated for each year. The results obtained are shown in Table 2. On a three years' average all treatments resulted in significant surplus yields compared to the control.

The double amount ($N_{150}P_{80}K_{100}$) autumn fertilization did not reach the efficiency of single dose ($N_{75}P_{40}K_{50}$) spring application. Compared to the nitrogen fertilizer given in autumn in a single dose, the divided autumn–spring application of the same amount of nitrogen provided a significantly larger grain yield. When nitrogen was distributed in autumn in a single dose there was no difference between the results of the different methods of working it in. In the case of a divided autumn — spring nitrogen application the result of incorporation by disk was tendentially better than that attained by ploughing under.

On the basis of a grain — straw ratio obtained from sample sheaves taken per plot (total yield of two metres in each) the straw yield was also calculated. The straw yield per year and on a three years' average is shown in Table 3. Differences in straw yield between the treatments increased with all treatments in the first year and decreased in the second and third year compared to the grain yields. Divided application of nitrogen gave significantly higher yields than autumn distribution in a single dose in the case of straw yield too. As to the method of incorporation of the fertilizer, in the case of single dose autumn application there was only a slight and naturally not significant difference between the results of the two methods of incorporation. In the case of divided nitrogen distribution the use of a disk for the incorporation of the fertilizer resulted in larger yields, but this difference was shown only in tendency.

In a highly calcareous low-fertility sandy soil the yields of rye can be multiplied by fertilization, though the absolute values are only 10—15 q/ha of grain and 15—20 q/ha of straw. Under the experimental conditions the average yield surplus attainable by a medium ($N_{75}P_{40}K_{50}$) rate of fertilization is 4.5 q/ha in grain and 6.2 q/ha in straw yield compared to the untreated control.

With the double amount of fertilizer — N_{150} (half in autumn, half in spring) P_{80}—K_{100}— the yield can be significantly increased by a further 5 q/ha grain and 15 q/ha straw. N_{75} distributed in spring ensures a larger yield than the double amount —N_{150} — applied in autumn only.

$P_{80}K_{100}$ and 150 kg/ha nitrogen distributed on two occasions (half in autumn, half as top dressing in spring) results in a significantly higher grain and straw yield than the same rate of fertilization with nitrogen applied in autumn.

In the case of applying a single dose of fertilizer in autumn there is no demonstrable difference between the results of incorporation by ploughing and disking. When nitrogen was distributed on two occasions (autumn and spring) the use of a disk for the incorporation of the fertilizer ensured each year a larger yield than when the fertilizer was ploughed under.

In highly calcareous poor sandy soils the growing of rye and its fertilization are mainly economic problems. It depends on the economic efficiency of production whether areas similar to that of the field of the trial should be utilized for field crops, forest or horticultural production.

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B. Lásztity

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IMPORTANCE OF CAROLUS CLUSIUS' LIFE-WORK IN THE HISTORY OF MYCOLOGY

Charles de L'Escluse (in its latinized form: Carolus Clusius) is an outstanding personality of the renaissance of botany in the 16th century. He explored the flora of Europe from Portugal to Hungary, his works represent the summit of descriptive botany. Over the past centuries Clusius has served as a valuable source for studies on the history of individual plant species, the time of their discovery or introduction as well as on the origin of many rare, interesting or lesser known plants.

The Belgian scientist, who studied botany under Rondelet in Montpellier, lived in Austria and Hungary between 1573 and 1588. Clusius' stay in Hungary was rendered possible by Boldizsár Batthyány, palatine of Hungary, hero of the days of Turkish rule, a generous patron of the sciences. As Batthyány's guest he found that mushrooms were commonly eaten in Hungary, and, led by his own curiosity, he began to occupy himself with practical mycology; with the help of local specialists he learned to make a distinction between edible and poisonous mushrooms. As a point of interest it may be mentioned here that he himself never consumed mushrooms and could not therefore give an account of their taste.

Prior to discussing the mycological merits of Clusius' work it should be noted that the taxonomical systems for plants and fungi, which he introduced and applied, have not proved lasting. The absence of a scientific systematization does not however detract from the value of Clusius' work, since the contemporary level of sciences was not capable of producing any more thorough classification. Clusius' contemporaries likewise discussed plants in a convenient succession or took over the classification used by the ancient Greek and Roman classics (as did Clusius in the case of fungi): e.g. J. Dalechamp, P. A. Mattioli, R. Dodonaeus and U. Aldrovandi (the latter distinguished between perfect and imperfect plants in his work "*Syntaxis plantarum*", that is, plants where either the leaf, the stem or the seed were missing were considered imperfect plants, and since he could not even find the fruit of the fungi he classified them as imperfect plants with a *Boletus* sp. as representative). Dodonaeus classified plants on the basis of an external character, simple or compound leaf, as Lobelius did. C. Gesner was the first to think of relationships between plants mainly on the basis of similar flowers, and to come close to the concept of genus. A. Cesalpino, who classified plants on the basis of root and fruit, is the representative of systematics and the "artificial" method of observation, in contrast to the "natural" method of Lobelius and J. Bauhin. Cesalpino adopted the view propounded by Aristotle, namely, that the essential character of plants is that they possess a vegetative "spirit" responsible for nutrition and reproduction. Since fungi did not possess (as far as contemporary knowledge was concerned) either roots or reproductive organs, Cesalpino placed them in his system between minerals and plants (that is, on the borderline of organic and inorganic matter).

From the point of view of botany the most valuable result of Clusius' stay in Hungary is his work "*Fungorum in Pannoniis observatorum brevis Historia*", which includes xylographs of 32 species drawn by Clusius. Clusius compiled this work in Leyden in 1597, on the basis of mycological studies carried out from 1584 onwards on Batthyány's estates in Vas and Zala counties, and with water-colours painted by a French painter invited from Vienna by Batthyány. (The paintings, together with the notes written by Clusius, form the "*Clusius codex*" of Leyden which was not published during the scientist's lifetime, but only at the beginning of the 20th century, when Gy. Istvánffi supplied the work with explanations and published it in 1900 at his own expense in a facsimile edition to celebrate the three hundredth anniversary of the publication of "*Rariorum Plantarum Historia*"). In four years the painter made a total of 221 pictures on 86 pages to which — besides other critical notes — Clusius wrote the Hungarian and German names with the help of Batthyány and I. Beythe.

Fig. 1. Carolus Clusius at the age of 60

It was published as an appendix to Clusius' "*Rariorum Plantarum Historia*" (Antwerp, 1601), the very first detailed monograph in the history of botany, presenting the fungal flora of a geographical unit, followed in the work by Chapter LXX of book X of G. B. della Porta's "*Villae libri XII*" (Naples, 1592) (this paper describes some fungi, mainly from the neighbourhood of Naples); finally, at the request of the printers, Clusius inserted as a further appendix some descriptions and pictures of fungi by Lobelius (previously published as the appendix to Lobelius' "*Herbaria Germanicae*" completed by some notes of his own.[1]

Prior to Clusius' work investigations concerning fungi were sporadic. The contemporary phytographists did no more than touch upon fungi, mentioning that they were strange and interesting organisms worthy of mention, either owing to their toxic nature or because of their utilization as food or for other purposes (as tinder, medicine, colouring material). Clusius was the first person in the history of botany to describe in a systematic work the fungi found in a particular floristic area (similar works were not written until the 18th century), and synthetized them into groups, relying purely on his own observations, since no other mycological literature was then available, and the world knew very little about these organisms.

Clusius described a total of 105 "species" (the mere fact that from the time of Dioscoride to Clusius the botanical works did not describe more than 40—50 species altogether points to the great progress made by Clusius in the history of mycology). The correctness of his

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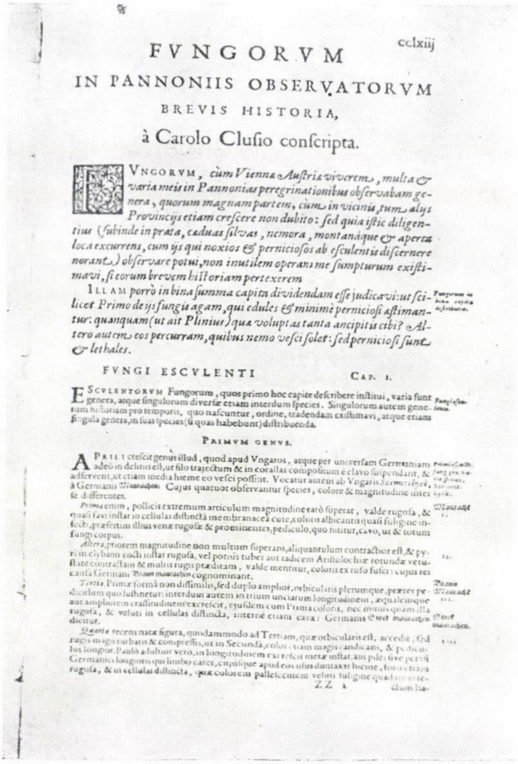
[1] To the description of *Phallus impudicus*, first given by L. H. Junius and adopted by Lobelius, Clusius added that he noticed the fungus in Amsterdam in 1597.



cclxiij

FVNGORVM
IN PANNONIIS OBSERVATORVM
BREVIS HISTORIA,
à Carolo Cluſio conſcripta.

FVNGORVM, cùm Viennæ Auſtriæ viverem, multa & varia meis in Pannonias peregrinationibus obſervabam genera, quorum magnam partem, cùm in vicinis, tum alijs Provincijs etiam creſcere non dubito: ſed quia iſtic diligentius (ſubinde in prata, cæduas ſilvas, nemora, montanáque & aperta loca excurrens, cum ijs qui noxios & pernicioſos ab eſculentis diſcernere norant) obſervare potui, non inutilem operam me ſumpturum exiſtimavi, ſi eorum brevem hiſtoriam pertexerem

ILLAM *porrò in bina ſumma capita dividendam eſſe judicavi: ut ſcilicet Primo de ijs fungis agam, qui edules & minimè pernicioſi æſtimantur: quanquam (ut ait Plinius) quæ voluptas tanta ancipitis cibi? Altero autem eos percurram, quibus nemo veſci ſolet: ſed pernicioſi ſunt & lethales.*

FVNGI ESCVLENTI CAP. I.

ESCVLENTORVM Fungorum, quos primo hoc capite deſcribere inſtitui, varia ſunt genera, atque ſingulorum diverſæ etiam interdum ſpecies. Singulorum autem generum hiſtoriam pro temporis, quo naſcuntur, ordine, tradendam exiſtimavi, atque etiam ſingula genera, in ſuas ſpecies (ſi quas habebunt) diſtribuenda.

PRIMVM GENVS.

Fig. 2. First page of Clusius "*Fungorum in Pannoniis observatorum brevis Historia*"

definitions can be checked up even today on the basis of the illustrations.[2] Clusius furnished the pictures with the names of the fungi and with various critical and other[3] observations.

The fungi described were grouped in genera each containing an average of 4—5 species. His classification was not, however, based on decisive, but only on secondary characteristics, such as the edible or poisonous nature of the fungi (this method was also used by Dioscorides and Pliny). Nevertheless, this does not detract from his merit, since the later systems of fungus classification were only given a scientific foundation after the discovery of the microscope. Clusius lists 46 edible and 59 poisonous muchrooms from Pannonia which are floristically characteristic of this region.

Knowledge of fungi prior to Clusius

In works preceding the time of Clusius, and even in those written by his contemporaries, fungi are only mentioned in passing.[4] Theophrastos, Dioscorides and classic Latin authors such as Pliny, Horace, Martial, Juvenalis and Apicis Caelius mentioned mushrooms (often without

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[2] Clusius, realizing that colour was the decivise character in identifying fungi and that it changed quickly during preservation, asked the painter to paint the collected specimens at once.

[3] In the case of edible mushrooms Clusius often described the manner of preparation too.

[4] It is almost certain that at the dawn of human life people gathering fruits chewed some kind of hemp, amanita and nightshade for their intoxicating sap. Yeast fungi (without

Fig. 3. G. B. della Porta (Naples 1535—1615), Italian naturalist

any scientific basis whatsoever), but this only proves that mushrooms were already known in the ancient world: e.g. *Boletus edulis* as an edible mushroom, and other "*Agaricus*, *Pezicae* and *Suillus*" species.[5]

Theophrastos mentioned the puff-ball. Dioscorides divided mushrooms into two groups: odible and poisonous mushrooms. His classification was adopted by Pliny, who placed mushrooms among the grasses and stated that they were exudations from trees and did not live longer than a year. He mentioned some edible mushrooms *(Fistulina hepatica, Macrolepiota procera, Boletus edulis, Amanita caesarea, Terfezia leonis, Agaricus campestris)*, and made a

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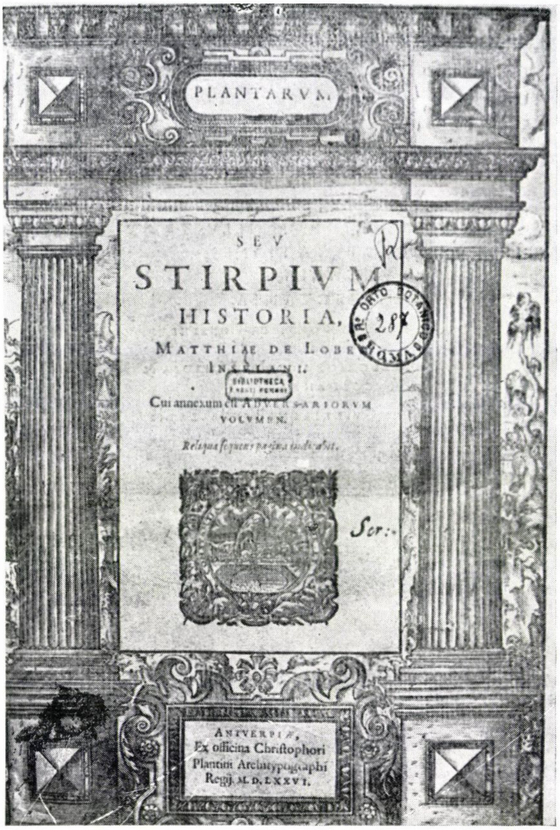
being identified) were used for bread-making even in Pliny's days (23—79 A. D.). The gardeners of Latin Rome took pride in growing champignons. In 934 an unknown disease appeared in the neighbourhood of Limoges. It was ergot poisoning; due to the propagation of *Claviceps purpurea* the bread consumed contained ergot. In the Middle Ages mushroom sauce was one of the favourite sauces, as mentioned by the "Parisian host", a book originating from the 14th century. Catherine Medici who arrived in France in 1535 introduced many Italian customs e.g. the consumption of truffles. In the middle of the 16th century muzzled swine were used in France to look for this mushroom, which — as the princess writes — "must be consumed as the last dish, because it helps in forwarding the meat from the pit of the stomach".

[5] *Amanita muscaria* is recorded in Asia (Kamchatka) as a narcotic of ancient times which was either swallowed without chewing or ased after drying; its intoxicating effect was stronger than that of any strong drink. The fairy-ring (e.g. the *Marasmius* and *Tricholomy* species) has long been the subject of popular superstition.



PLANTARVM

SEV
STIRPIVM
HISTORIA,
MATTHIAE DE LOBE
INSVLANI.
Cui annexum est ADVERSARIORVM
VOLVMEN.
Reliqua ſequens pagina indicabit.

Scr:.

ANTVERPIAE,
Ex officina Chriſtophori
Plantini Architypographi
Regij. M.D.LXXVI.

Fig. 4. Title-page of Matthias Lobelius' (M. de Lobel) "*Plantarum seu Stirpium Historia*" published in Antwerp 1576

morphological distinction between "root, stem and cap" *("Pezicae sine radice aut pediculo nascuntur")*.

Even later only a few species were described (without any kind of systematization, but simply supplied with a commonly used name); they were of practical use mainly as medicines. The fact that the same species (or mushrooms thought to belong to the same species) were sometimes poisonous, and sometimes consumed without any ill effects, created the belief that mushrooms were not poisonous in themselves but only became poisonous through special circumstances, such as the vicinity of certain trees, serpent's nests, rusty iron, etc. Dioscorides and Paulus Aegineta[6] considered mushrooms to be harmful to man under any circumstances owing to their poisonous nature and low digestibility. Avicenna[7] too warned the public against mushroom consumption. *Amanita phalloides*, *A. caesarea* and *A. muscaria*, as well as the truffle, were mentioned by Galen, who considered every mushroom more or less poisonous. *Boletus edulis* was also mentioned by Nicander, while Columella knew the *Morchella esculenta*. *Polyporus*

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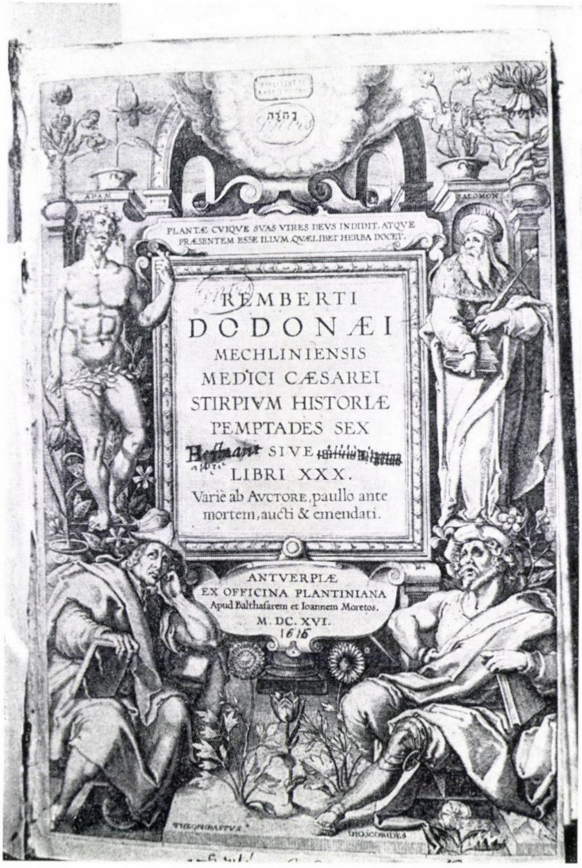
[6] This scientist of Greek extraction lived from 615 to 690; he wrote about *Fomes fomentarius* and its practical use.

[7] The Arab scientist lived from 979 to 1037. His main work was "*Liber canonis . . .*".



PLANTÆ CVIQVE SVAS VIRES DEVS INDIDIT, ATQVE PRÆSENTEM ESSE ILLVM QVÆLIBET HERBA DOCET.

REMBERTI
DODONÆI
MECHLINIENSIS
MEDICI CÆSAREI
STIRPIVM HISTORIÆ
PEMPTADES SEX
SIVE
LIBRI XXX.
Variè ab AVCTORE, paullo ante
mortem, aucti & emendati.

ANTVERPIÆ
EX OFFICINA PLANTINIANA
Apud Balthasarem et Ioannem Moretos.
M. DC. XVI.
1616

Fig. 5. Title-page of Rembert Dodonaeus' "*Stirpium Historia Pemptades*", from a copy made in Antwerp 1616

igniarius, P. fomentarius, Daedelia quercina have been known since Roman times. Mushrooms were mentioned by Oribasius of Pergamo and d'Amida as well.

Between the 8th and 15th centuries studies on mushrooms — like all natural sciences — were thrust into the background. From this age the names of the Abbess Hildegard and Albertus Magnus should be mentioned; the latter thought mushrooms to be exostoses of plants. He was the first to describe the fly-killing effect of *Amanita muscaria*.

A chronological list of authors who discussed mushrooms in their works must include B. Rinius, whose book "*Liber de simplicibus*" was published in Venice in 1415. J. Mesué writes about mushrooms in his work published in Venice in 1487. In "*Hortus sanitatis*" (Mainz, 1485) the drawing of an "*Agaricus*" species can be found. Ermolao Barbarp[8] was the author of "*Castigationes Plinianae*", published in Rome in 1492, which is a translation of studies by Pliny and Dioscorides (to which he added five books entitled "*Corollari*" containing corrections and explanations). He divided mushrooms into the following categories according to their shapes: "egg-shaped, sponge-like, finger-like, peziza, plum-shaped, needle-like, heart-shaped,

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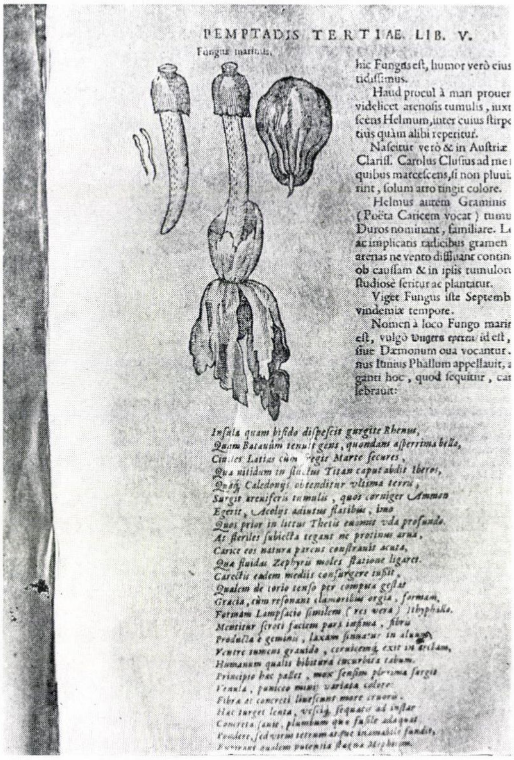
[8] Born in Venice, lived from 1454 to 1492.



PEMPTADIS TERTIAE. LIB. V.

Fungus marinus.

hic Fungus eſt, humor verò eius
tidiſſimus.
Haud procul à mari prouer
videlicet arenoſis tumulis, iuxt
ſcens Helmum, inter cuius ſtirpe
tiùs quàm alibi reperitur.
Naſcitur verò & in Auſtriæ
Clariſſ. Carolus Cluſius ad me
quibus marceſcens, ſi non pluui
rint, ſolum atro tingit colore.
Helmus autem Graminis
(Poëta Caricem vocat) tumu
Duros nominant, familiare. L
ac implicans radicibus gramen
arenas ne vento diffluant contin
ob cauſſam & in ipſis tumulor
ſtudioſè ſeritur ac plantatur.
Viget Fungus iſte Septemb
vindemiæ tempore.
Nomen à loco Fungo mari
eſt, vulgò [illegible] id eſt,
ſiue Dæmonum oua vocantur.
nus Iunius Phallum appellauit,
ganti hoc, quod ſequitur, ca
lebrauit:

Inſula quam bifido diſpeſcit gurgite Rhenus,
Quam Batauûm tenuit gens, quondam aſperrima bello,
Ciuiles Latias cùm fregit Marte ſecures,
Qua nitidum in fluctus Titan caput abdit Iberos,
Quàq; Caledonijs obtenditur vltima terris,
Surgit areniferis tumulis, quos corniger Ammon
Egerit, Aeolijs adiutus flatibus, imo
Quos prior in littus Thetis euomit vda profundo.
At ſteriles ſubiecta tegant ne protinus arua,
Carice eos natura parens conſtrauit acuta,
Quæ fluidas Zephyris moles ſtatione ligaret.
Carectis eadem medijs conſurgere iuſſit,
Qualem de corio tenſo per compita geſtat
Græcia, cùm reſonant clamoribus orgia, formam,
Formam Lampſacio ſimilem (res vera) Ithyphallo.
Mentitur ſcroti faciem pars infima, fibris
Producta è geminis, laxam ſinuatur in aluum,
Ventre tumens grauido, ceruicemq; exit in arctam,
Humanum qualis bibitura cucurbita tabum.
Principio hæc pallet, mox ſenſim plurima ſurgit
Venula, puniceo minij variata colore.
Fibra at concreti liueſcunt more cruoris.
Hæc turget lenta, veſicaq; ſequatur ad inſtar
Concreta ſanie, plumbum quæ fuſile adæquat
Pondere, ſed virus tetrum atque inamabile fundit,
[illegible] qualem putentia ſtagna Mephitim.

Fig. 6. Phallus impudicus, taken from Dodonaeus' work (with reference to Clusius' work)

tinder mushroom, stone mushroom, etc." (that is, *Amanita, Clavaria, Lepiota, Polyporus frondosus, Sparassis crispa, Clitopilus prunulus, Tricholoma georgii, Pleurotus eryngii, Polyporus tuberaster, P. squamosus, Daedalea quercina*). The Frenchman J. Ruelle[9] writes in his work "*De natura Stirpium libri tres*" published in Basel (1536): "truffle is supposed to take its origin from a seed-like form", and that it is the wind and rain that start the development of mushrooms; further on he describes the developmental phases of *Amanita.*

In his works "*Historia Stirpium*" (1553) and "*Annotationes in Pedacii Dioscoridis*" (Frankfurt), 1549 the German V. Cordus[10] interprets the ideas of Dioscorides and divides mushrooms into two groups: harmful and harmless; he also describes the stimulatory effect of *Elaphomyces granulatus* on the sexual instinct.

In Italy Brassavola[11] deals with mushrooms in his book "*Examen simplicium medicamentorum*" published in Rome in 1536.[12] The Swiss L. Fuchs[13] mentions some mushroom species

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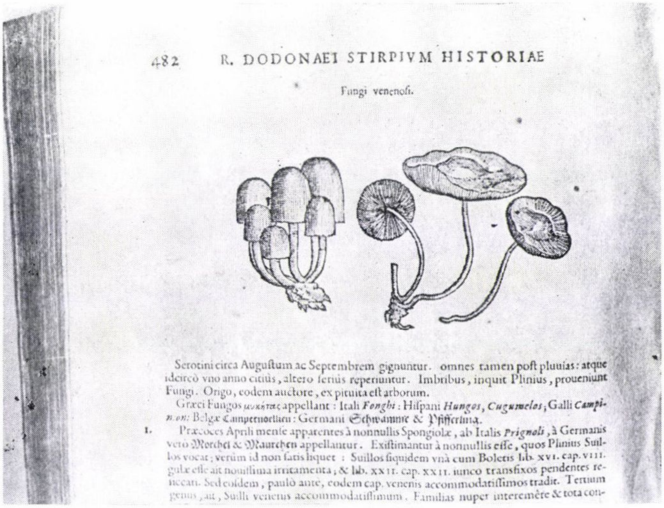
[9] Born in Soisson in 1479, died in Paris in 1539. He was professor at the medical faculty of the University of Paris.

[10] Born in 1515, died in 1544 in Rome. Cordus and Clusius were given the name "*patres pharmacognosiae*" (fathers of pharmacology) by the historians; Cordus earned this title with his work "*Dispensatorium simplicium*", published in Nürnberg in 1546.

[11] Born in Ferrara in 1500, died in 1555.

[12] It is illustrative of the confusion which existed concerning mushrooms that Rinius, Brassavola and Fuchs, and much later even Boccone, thought that the parasite *Cytinus hypocistis* on *Cistus salviaefolius* was a mushroom; P. A. Micheli, Clusius' contemporary,



482 R. DODONAEI STIRPIVM HISTORIAE

Fungi venenoſi.

Serotini circa Auguſtum ac Septembrem gignuntur. omnes tamen poſt pluuias: atque idcircò vno anno citiùs, altero ſeriùs reperiuntur. Imbribus, inquit Plinius, proueniunt Fungi. Origo, eodem auctore, ex pituita eſt arborum.

Græci Fungos μυκητας appellant: Itali *Fonghi*: Hiſpani *Hungos, Cugumelos*; Galli *Campi-gnon*: Belgæ Campernoellien: Germani Schwamme & Pfifferling.

1\. Præcoces Aprili menſe apparentes à nonnullis Spongiolæ, ab Italis *Prignoli*, à Germanis verò Morchel & Maurchen appellantur. Exiſtimantur à nonnullis eſſe, quos Plinius Suillos vocat; verùm id non ſatis liquet: Suillos ſiquidem vnà cum Boletis lib. XVI. cap. VIII. gulæ eſſe ait nouiſſima irritamenta; & lib. XXII. cap. XXII. iunco transfixos pendentes ſiccari. Sed eoſdem, paulò ante, eodem cap. venenis accommodatiſſimos tradit. Tertium genus, ait, Suilli venenis accommodatiſſimum. Familias nuper interemère & tota con-

Fig. 7. Poisonous fungi, taken from Dodonaeus' work

in "*Historia Stirpium*" (1542). His friend and compatriot K. Gesner[14] reports on "*Fungus ramosus*" *(Clavaria coralloides)*. Mushrooms are also mentioned by J. Gesner in "*Tabulae phytographicae*". H. Cardanus, mathematician and travelling naturalist,[15] refers to "*Agaricus*" in his work on exotic plants, published in 21 books in Nürnberg in 1550.

In his "Kräuterbuch" *("Hieronimi Tragi De Stirpium . . .*", published in Strassburg in 1552, H. Bock[16] describes a total of 12 fungus species, and as to the origin of fungi he writes: "from this moisture (that is, from the moisture of the soil) come all truffles and mushrooms". In "*Historia Stirpium*" (1560) he writes that the fungi are neither plants, nor roots, nor flowers, nor seeds, but are products of the moisture of the earth — as taught by Aquinas Ponta —, and that fungi produce no seed. He describes *Clavaria coralloides*, for example, in the following way: "*Fungi digitelli, partim lutei, partim ex albo pallidi*".

A. Lonicerus[17] reports on mushrooms in "*Naturalis historiae opus*", published in 1551 (e.g. he describes truffles — like Mattioli — as "*fungi cervini sive Cervi Boletus*"). The Englishman W. Turner, who was a disciple of L. Ghini in Bologna and died in 1568, speaks of mushrooms in his work "*Herba britannica*".[18]

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recognised it as a phanerogamous organism, while Mattioli compared it to *Orobanche* and excluded the possibility of its being a fungus.

[13] Born in Wemdingen in 1501, died in Tübingen in 1566. The first attempt at a botanic nomenclature was made by this Munich physician.

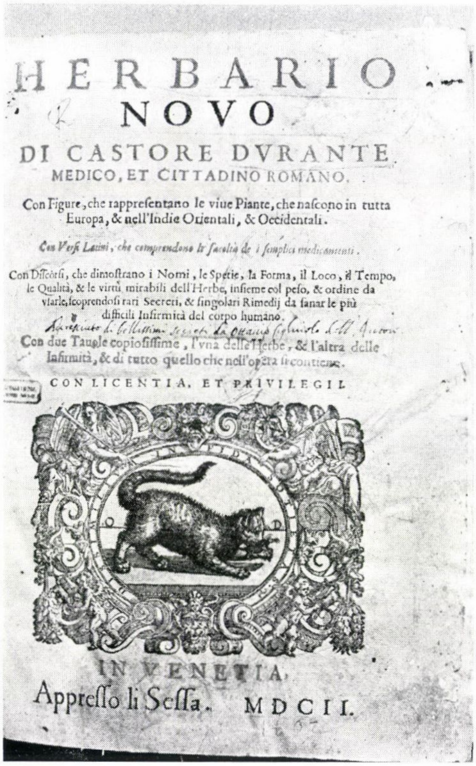
[14] Born in Zurich in 1516, died in Basel in 1565. He was a physician and professor who toured about France and Italy where he became friendly with Mattioli. Mattioli and others used drawings from Gesner's "*Opera botanica*" in their works.

[15] Born in Pavia in 1501, lived as a physician in a number of Italian towns, travelled in England, and died in Rome in 1576.

[16] Alias Tragus. Born in Heiderbach in 1498, died in 1554. He learned theology and medicine from O. Brunsfeld; later, as warden of the local botanical garden, he wrote the "*Herbarium vivae eicones*" between 1530 and 1536 and the "New Kreuter Buch" in 1539.

[17] Born in Marburg in 1527, professor of mathematics and physics in Frankfurt.

[18] In his "New herball" (London, 1568) Turner (1515—1568) no longer places the species in alphabetical order but discusses them on the basis of their different characteristics.



HERBARIO
NOVO
DI CASTORE DVRANTE
MEDICO, ET CITTADINO ROMANO.
Con Figure, che rappreſentano le viue Piante, che naſcono in tutta Europa, & nell'Indie Orientali, & Occidentali.
Con Verſi Latini, che comprendono le facoltà de i ſemplici medicamenti.
Con Diſcorſi, che dimoſtrano i Nomi, le Spetie, la Forma, il Loco, il Tempo, le Qualità, & le virtù mirabili dell'Herbe, inſieme col peſo, & ordine da vſarle, ſcoprendoſi rari Secreti, & ſingolari Rimedij da ſanar le più difficili Infirmità del corpo humano.
Accresciuto di bellissimi secreti da Ottavio figliuolo dell'Autore
Con due Tauole copioſiſſime, l'vna delle Herbe, & l'altra delle Infirmità, & di tutto quello che nell'opera ſi contiene.
CON LICENTIA, ET PRIVILEGII.
IN VENETIA,
Appreſſo li Seſſa. MDCII.

Fig. 8. Title-page of Castoro Durante's "*Herbario novo*" from a copy published in Venice, 1602

In his work "*Commentarii in Pedacii Dioscoridis*" (Venice, 1554) the Italian P. A. Mattioli[19] completes his explanation of Dioscorides' theses with a description of *Tricholoma georgii*, *Amanita caesarea*, *Pleurotus eryngii*, *R. ostreatus*, *P. olearius*, *Polyporus sulphureus*, *Fomes officinalis*, *Rhizopogon*, *Lycoperdon*, *Clavaria* and truffle species, using their common names.

H. Junius,[20] a Dutch physician (and educated historian, who spoke seven languages), who travelled in Spain, France, Germany, Italy and England, published a monograph entitled "*Phalli, ex fungorum genere in Hollandiae*" in Delft in 1564, in which he presents *Phallus* as a object of curiosity occurring exclusively in the Netherlands, describing the shape and special features of the mushroom as well as its utilization in curing chronic arthritis and gout.[21] This fungus species was also known to his contemporaries Clusius, Mattioli,[22] Dodonaeus and Lobe-

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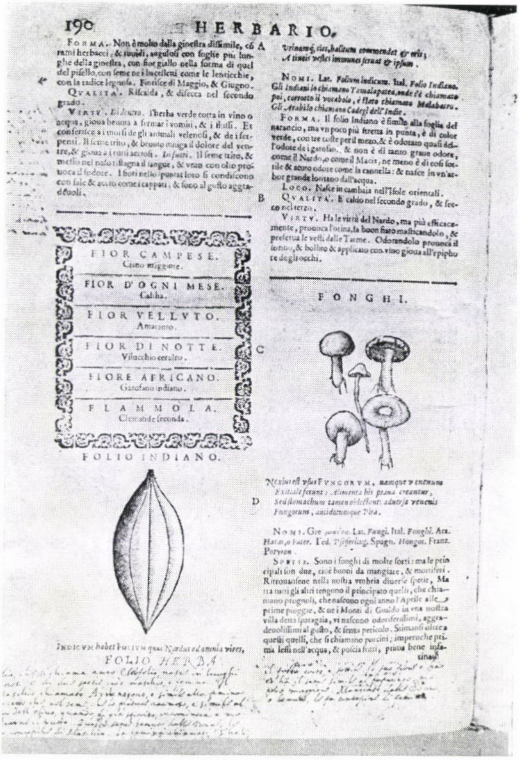
[19] Born in Siena in 1500, died in Trento in 1577.

[20] Alias H. Jonghe. Born in Horna in 1512, died in Middelburg in 1575.

[21] N. Espillet, physician, (who became acquainted with Aldrovandi in the course of a journey to Rome and Bologna) mentioned in a letter written to Aldrovandi from Lille on 12th June 1567 that he had sent his fellow scientist a description of *Phallus* taken from Junius' book. Bauhin adopted Junius' full description of *Phallus* in the "*Pinax*" (Basel, 1623), as Dodonaeus did in the "*Pemptades*".

[22] He mistook it, however, for "*Satirion*", which was mentioned by Dioscorides; this has three leaves, and is thus totally different.



190 HERBARIO.

Forma. Non è molto dalla gineſtra diſſimile, cõ rami herbacei, & ruuidi, anguloſi con foglie più lunghe della gineſtra, con fior giallo nella forma di quel del piſello, con ſeme ne i baccelletti come le lenticchie, con la radice legnoſa. Fioriſce di Maggio, & Giugno.

Qualità. Riſcalda, & diſecca nel ſecondo grado.

Virtù. *Di dentro.* L'herba verde cotta in vino o acqua, gioua beuuta a fermar i vomiti, & i fluſſi. Et conferiſce a i morſi de gli animali velenoſi, & de i ſerpenti. Il ſeme trito, & beuuto mitiga il dolore del ventre, & gioua a i tumori acceſi. *Di fuori.* Il ſeme trito, & meſſo nel naſo riſtagna il ſangue, & vnto con olio prouoca il ſudore. I fiori nello ſpuntar loro ſi condiſcono con ſale & aceto come i cappari, & ſono al guſto aggradeuoli.

FIOR CAMPESE.
Ciano maggiore.

FIOR D'OGNI MESE.
Caltha.

FIOR VELLVTO.
Amaranto.

FIOR DI NOTTE.
Vilucchio ceruleo.

FIORE AFRICANO.
Garofano indiano.

FLAMMOLA.
Clematide ſeconda.

FOLIO INDIANO.

Indicvm habet Folivm quas Nardus ad omnia vires,
FOLIO HERBA

Vrinamq; ciet, halitum commendat & oris,
A tineis veſtes immunes ſeruat & ipſum.

Nomi. Lat. *Folium Indicum.* Ital. *Folio Indiano. Gli Indiani lo chiamano Tamalapatra, onde s'è chiamato poi, corrotto il vocabolo, è ſtato chiamato Malabatro. Gli Arabi lo chiamano Cadegi dell'Indie.*

Forma. Il folio Indiano è ſimile alla foglia del narancio, ma vn poco più ſtretta in punta, è di color verde, con tre coſte per il mezo, & è odorato quaſi dell'odore de i garofani, & non è di tanto graue odore, come il Nardo, o come il Macis, ne meno è di coſi ſottile & acuto odore come la cannella: & naſce in vn'arbor grande lontano dall'acqua.

Loco. Naſce in Cambaia nell'Iſole orientali.

Qualità. È caldo nel ſecondo grado, & ſecco nel terzo.

Virtù. Ha le virtù del Nardo, ma più efficacemente, prouoca l'orina, fa buon fiato maſticandolo, & preſerua le veſti dalle Tarme. Odorandolo prouoca il ſonno, & bollito & applicato con vino gioua all'epiphore degli occhi.

FONGHI.

Noxius eſt vſus Fvngorvm, *namque venenum*
Exitiale ferunt: Alimenta his praua creantur,
Sed ſtomachum tamen oblectant: aduerſa venenis
Fungorum, antidotumque Vira.

Nomi. Gre. [illegible] Lat. *Fungi.* Ital. *Fonghi.* Ara. *Hatar, o Fater.* Ted. *Tiſſerling.* Spagn. *Hongos.* Franz. *Potyron.*

Spetie. Sono i fonghi di molte ſorti: ma le principali ſon due, cioè buoni da mangiare, & mortiferi. Ritrouanſene nella noſtra Vmbria diuerſe ſpetie. Ma tra tutti gli altri tengono il principato quelli, che chiamano prugnoli, che naſcono ogni anno l'Aprile alle prime pioggie, & ne i Monti di Gualdo in vna noſtra villa detta ſparagaia, vi naſcono odoriferiſſimi, aggradeuoliſſimi al guſto, & ſenza pericolo. Stimanſi oltre a queſti quelli, che ſi chiamano porcini; imperoche prima leſſi nell'acqua, & poſcia fritti, prima bene infarinati

Fig. 9. Picture of a mushroom, taken from-Durante's work

lius[23] and many other authors found it in their own countries, so the exclusive occurrence off *Phallus* in Holland was soon disproved.

The famous U. Aldrovandi[24] also dealt with mushrooms, as shown by seven paintings found in his herbarium (one of them is of *Boletus cervinus*) and by his correspondence with C. Felici, which suggests that the Rimini physician sent Aldrovandi the "*Lectio de fungis*" in 1565. Regrettably enough, in his work "*Dendrologia*", published in Bologna in 1668, O. Montalbano (i.e. A. Bumaldi) made rather a bad business of revising Aldrovandi's notes on fungi which were not published during the scientist's lifetime. The obscure and scientifically unacceptable notes suggest that Montalbano, who was far less talented than Aldrovandi, attributed too many of his own ideas to the scientist when interpreting his manuscript "*Historia Arboribus*". In spite of all this, the work testifies to the fact that Aldrovandi was acquainted with the species *Fomes officinalis*, *Fistulina hepatica*, *Daedalea quercina*, *Gloeophyllum sepiarium*, *Armillaria mellea*, *Pleurotus ostreatus*, *Trametes cinnabarina*, *Ganorerma lucidum*, *Sarcoscypha coccinea*.

A. Ciccarelli wrote a monograph on truffle entitled "*Opusculum de tuberibus*" (Padua, 1564) indicating the site of occurrence (environs of Rome, etc.). He also mentions "*pietra fungaia*" (mushroom bed stone, i.e. *Polyporus tuberaster*), which his father-in-law must have

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[23] He mentions having encountered specimens of *Phallus* in Antwerp from 1559 onwards; ording to Dodonaeus, Junius found the mushroom as early as 1541.

[24] The physician-scientist of Bologna lived from 1522 to 1605.

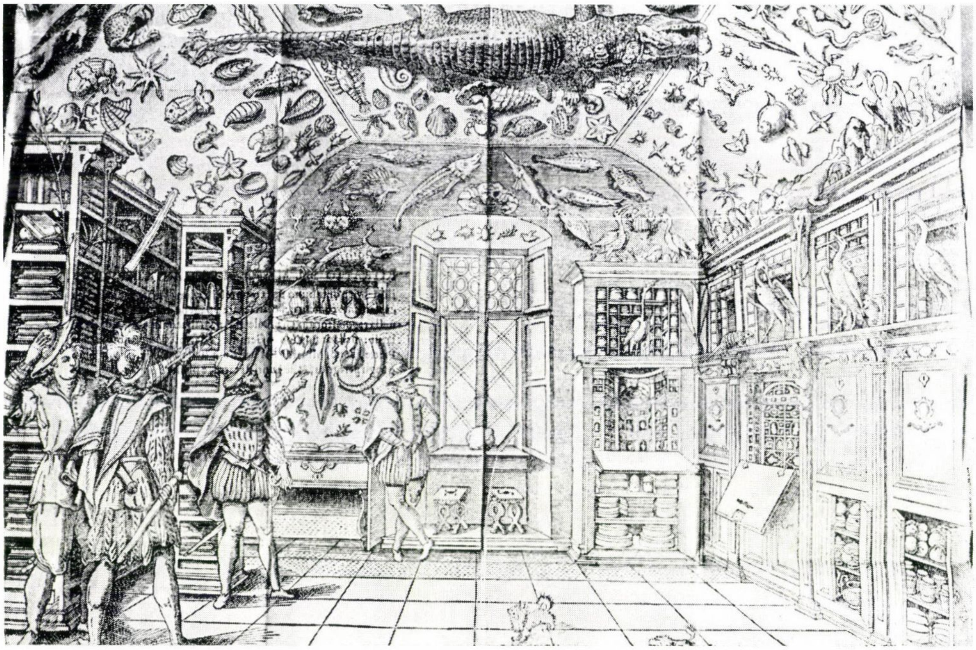

Fig. 10. Ferrante Imperato's Museum of "natural sciences" in Naples, from the book by his son Francesco Imperator "*Discorsi intorno a diverse cosi naturali*" published in Naples, 1628

found in the neighbourhood of Rome and Naples. B. Sacci also writes about mushrooms in "*Historiae Ticinensis Libri X*", published in 1565. In "*Farmacopea Bergamasca*", under the heading "*De usitatiorum medicamentorum*", an, "*Agaricum*" *(Fomes officinalis)* is described together with its therapeutical utilization.

R. Dodonaeus and M. Lobelius, compatriots and friends of Clusius, refer to mushrooms in their botanical works. Among others M. Lobelius[25] reports on *Phallus impudicus*, already described by H. Junius, as "*Fungus virili panis erecti facies*". In his "*Fungorum Historia*" Clusius himself used two pictures, one of *Morchella esculenta*, the other of *Bovista nigrescens*, taken from Lobelius' "*Icones*"; and at the request of the typographer, as an appendix he translated from Flemish into Latin the descriptions of some fungi from Lobelius' "*Kruydtboeck*" and "*Icones*", using the original pictures and adding notes of his own.

R. Dodonaeus (1517–1585) was court-physician to Maximilian II in Vienna between 1576 and 1579, and then, from 1582 onwards, professor of medicine in Leyden. In his work "*Pemptades seu Stirpium Historia*" (Antwerp, 1583 and 1612) he took on a complete study of H. Junius. (F. Imperato gave an account of Dodonaeus' species "*Kylü terrestris*" in 1610).

Of Clusius' Italian contemporaries who were also engaged in studies on mushrooms the most outstanding was A. Cesalpino,[26] who succeeeded L. Ghini as warden of the botanical

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[25] Born in Lille in 1538, died in 1616. Like Clusius, Dalechamp, etc. he was a disciple of Rondelet in Montpellier and later a physician, first in Antwerp and then in London (in the court of James I). He travelled in Germany and Italy. Among his other works are "*Stirpium Observationes*", "*Nova Stirpium Adversaria*" (with the collaboration of the Englishman P. Pena) and "*Plantarum seu Stirpium Historia*" (Antwerp, 1576).

[26] Born in Arezzo in 1525, died in Rome in 1603.

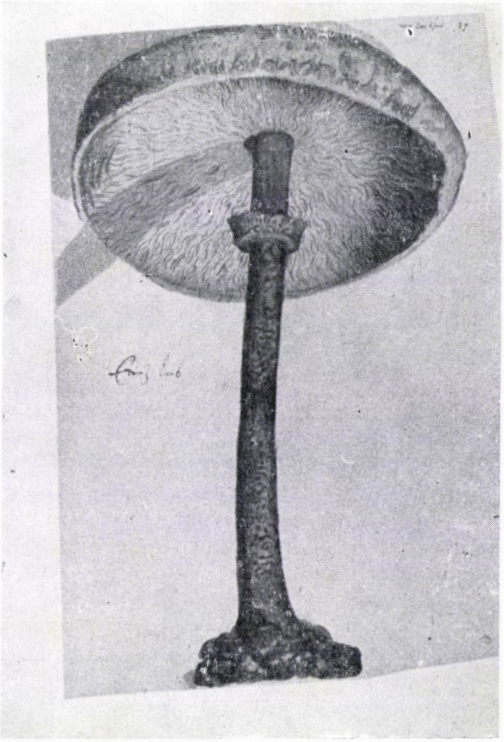

Fig. 11. Water-colour of Clusius' "XVIII. Genus esculentorum" *(Macropeliota procera)* on Plate 58 in the Clusius Codex, indicating the Hungarian name of the fungus "Ewz láb"

garden at Pisa in 1556.[27] In 1592, when G. Mercuriale was invited to be instructor at the university of Pisa, Cesalpino moved to Rome, where he lived for 11 years and became friendly with M. Mercati, founder of the botanical garden in the Vatican. Cesalpino was a naturalist of note, and an educated physician and physiologist. Imbued with philosophy, mainly with Aristotle's doctrines, he always endeavoured to co-ordinate the facts he studied; he was the first to give minerals a more rational classification, and the first to realize the necessity of applying a system to botany and of dividing the plants into trees, shrubs and grasses, and within these categories into genera. He did not, however, remain immune to the misconceptions of his age, especially concerning spontaneous generation, which he also accepted in the case of fungi, though he acknowledged the role of spores in the case of pteridophytes. As physician to Pope Clement VIII he described and systematized the fungi in his work "*De plantis liber XVI*" (Florence, 1583); book XVI contains the cryptomagous species: in order 4 of class 15 sixteen genera of fungi are listed under the designation "*herba sine semine*". His classification was based on the "*methodus a receptaculo*". He described the following species and "*genera*": "*Tuber*" (*Tuber* sp.), "*Pezicae*" *(Lycoperdon gemmatum)*, "*Fungus*": "*Fucco silvestre*" *(Clathrus cancellatus)*, "*Boletus*" (*Amanita* sp.), "*Suillus*" *(Boletus luridus, B. edulis)*, "*Prunulus*" *(Clitopilus)*, "*Prataeolus*" *(Coprinus)*, "*Famigliola*" *(Armillaria mellea)*, "*Scoroglia*" *(Macrolepiota procera)*, "*Fungus marinus*", "*Gallinaceus*" *(Cantharellus cibarius)*, "*Fungus panis*

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[27] In Pisa he was visited by P. Belon, M. Lobelius, G. Pona and Aldrovandi.

similis", "*Lingua*" *(Fistulina hepatica)*, "*Digitellus*" *(Clavaria)*, "*Ignarius*" *(Fomes fomentarius)*, "*Agaricum*" *(Russula olivascens, Fomes officinalis)*, "*Prugnoli*" *(Tricholoma georgii)*, "*Prataioli*" *(Psalliota)*, "*Mushroom bed stone*" *(Polyporus tuberaster)*.

In his work "*Herbario novo*" (Venice, 1584; Rome, 1585) C. Durante of Viterbo also discusses mushrooms. In subsequent editions of his book (Venice, 1602 and 1667) mushrooms are already dealt with on the basis of Clusius' work. In his opinion mushrooms are "nothing else than the superfluous moisture of earth, trees, decaying wood and unclean materials". He divides mushrooms according to their edible or poisonous nature. In the case of edible mushrooms he describes some methods of preparation, and recognizes the poisonous ones by their changed colour. He mentions an "*Agaricus*" living on *Larix decidua*, and, in connection with *Amanita muscaria*, speaks of its fly-killing effect. About "*Fungo Corraloide*" he writes: "it is supported by and takes up nutrients with thin, white, fibrous roots".

Of the other phytographers prior to Clusius who dealt with mushrooms mention must be made of J. Dalechamp,[28] who describes H. Junius' mushroom under the name "*Phallus batavicus*" in his work "*Historia Generalis Plantarum*" (Lyon, 1587).[29] According to Clusius the Neapolitan G. B. della Porta took over many data from Dalechamp for his work "*Villae*". J. Thalius[30] touches upon mushrooms in his book entitled "*Sylva hercina sive catalogous plantarum*" (Frankfurt, 1588). J. Th. aus Bergzabern (i.e. *Tabernaemontanus*),[31] physician, also discusses mushrooms in his works "*Eicones plantarum*" (1590) and "New volkommen Krauterbuch (his books contain many pictures taken from Clusius, Fuchs, Lobelius, Dodonaeus and Mattioli).

G. Mercuriale,[32] physician, was professor of medicine at the university of Padova between 1569 and 1586. He was a friend of Aldrovandi, in Bologna, and of professor G. B. Balestri, and also taught at the university of Bologna. In his work "*Rariorum lectiorum de medicinae*" (Venice, 1571) he mentioned mushrooms among the medicinal plants. He adopted the views professed by Theophrastos and Galen about mushrooms being rootless plants.

The naturalist and physicist G. B. della Porta,[33] who was the founder of the "*Accademia dei segreti*", the organizer in Naples of a botanical garden and a museum of objects falling within the scope of natural sciences, and later a founder member of the "*Accademia dei Lincei*" in Rome,[34] also dealt with mushrooms in the course of his botanical studies. In Naples he published the works "*Phytognomonica*" in 1588 and „*Villae libri XII*" in 1592. In the appendix of Clusius' "*Fungorum Historia*" we can find the chapter on mushrooms from della Porta's work "*Villae libri XII*" under the heading "*De Fungis, Caput LXX. Libri X. Villae Joan. Baptista Portae Neapolitani*", in which 20 species, including "boleti", "suilli", "conocchielle" *(Macrolepiota procera)*, "gallinaccia" *(Polyporus frondosus)*, "gallinella" *(Clavaria)*, "piperitis", "peperella" *(Lactarius piperatus)*, "richione" *(Pleurotus eryngii)*, "galucci" *(Hydnum repandum)*, "spongiole" *(Morchella)*, "monacelle", "virni", "prigniolo", "prunello", "spinullo", "ovoli" *(Amanita caesarea)*,[35] are listed by the author (Clusius often cites della Porta in "Fun-

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[28] Born in Caen in 1513, died in Lyon in 1588.

[29] The Lyon physician wrote this work together with J. Desmoulins.

[30] Alias Johann Thal (1542—1583).

[31] Lived from 1520 to 1588. He was H. Bock's pupil.

[32] Born in Forli in 1530, died in 1606.

[33] Born in Naples in 1540, died in 1615.

[34] The Academy was founded in 1601 by Prince F. Cesi who himself was interested in the natural sciences and also dealt with mushrooms. His "*Codice*" contains 600 pictures each showing 2 or 3 mushrooms collected mainly in the environs of Rome, and is supplied with an index.

[35] From the fact that Clusius took over the chapter on mushrooms from della Porta's book as an appendix, O. Comes and later G. B. Traverso (1905) drew the false conclusion that Clusius' "*Fungorum Historia*" was nothing more than a copy of the work published by della Porta in 1592. Such a conclusion can only be arrived at through a superficial knowledge

gorum Historia" itself when indicating the Italian locality of fungi which he found in Pannonia — e.g. Monte Gargano, Vesuvius, Sorrento, Afellino, Puglia, etc. — and their common Italian names — e.g. silli, conoccielle, gallinaccia, etc.). Della Porta's intuition concerning the reproduction of fungi is very interesting: "as opposed to the antique authors I state that every plant has seed, . . . even from the mushrooms I was able to isolate small black seeds found at the edge of the cap; thus in the case of the mushroom bed stone *(Polyporus tuberaster)* the mushrooms do not come from the stone, but through the continuous falling of mushroom seeds the stone becomes fertile. I have often seen mushrooms growing in places where the refuse of mushrooms used for consumption is thrown".

F. Colonna[36] followed the ideas of Gesner and Cesalpino in his mycological studies. In his work "*Echphrasis*" (Rome, 1606) the author of "*Phytobasanos*" (Naples, 1592) describes and illustrates the following species: *Peziza, Agaricus procerus, Clathrus cancellatus, Pleurotus eryngii, Sarcosphaera coronaria, Guepinia, Pleurotus ostreatus, Macrolepiota procera.*

Besides G. Pona, Ferrante Imperato, the Italian pharmacist, likewise dealt with mushrooms. In his natural science museum —as in that of Pona — mushrooms were also displayed, as shown by the book written by his son, Francesco Imperator: "*Discorsi intorno a diverse cose naturali*" (Naples, 1628).[37] In his work "*Dell'historia naturale*" (Naples, 1599) Ferrante Imperato describes the shape and taste of mushrooms he had observed and mentions their common names (e.g. spongiola, rugoso, ramoso, borsaro, vessicchia, etc.); he describes ten species in all, the most characteristic of which are: *Morchella, Clavaria, Lycoperdon, Boletus luridus, Polyporus tuberaster* and *Daedalia quercina.*

The Bauhin brothers' work — considering that it followed that of Clusius chronologically — will not be analysed here. When describing some 130 species in his book "*Pinax Theatri Botanici*" (1623) K. Bauhin followed the path laid down by Clusius (with differences only in classification; some fungi taken over from Clusius are mentioned as "*Fungi ungarici*"); in his work "*Historia plantarum universalis*" (1650) J. Bauhin proposes anew Clusius' xylographs (he, too, adopts H. Bock's views about the origin of mushrooms, namely, that they are neither plants, nor roots, nor flowers, nor seeds, but products of the moisture of earth, trees and decaying wood).

A review of the literature on fungi prior to, and contemporary with,Clusius shows that altogether these works did not describe more than 40—50 species, thus the sudden change in the number of known fungi was due to Clusius.

Identification of Clusius' Pannonian fungi

The work "*Fungorum in Pannoniis observatorum brevis Historia*", dated 1598, begins with a dedication to Pinelli.[38] In this dedication Clusius wrote he had mentioned to Pinelli the

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of Clusius' work, since after 1584 Clusius described Pannonian mushroom species that della Porta could not possibly have known, while on every occasion when he refers to della Porta's data Clusius also mentions his name. Della Porta was a clever and talented naturalist, physicist, chemist, etc., and often had bizarre ideas, but he never came up to Clusius' standard, particularly where mycology was concerned.

[36] Born in Naples in 1567, died in 1650.

[37] "Petrified sea mushrooms which Fabio Colonna in his wise book '*De purpurea*' Folio 33 names as fungi lapidei coralloides, . . . our museum keeps some terrestrial mushrooms which have become petrous and stripes appeared on their lower surfaces."

[38] Pinelli was born in Naples in 1535, where later he established a private park for botanical purposes; in 1558 he moved to Padua where he organized a library and a museum of natural sciences. His services were higly valuable to Clusius since he was a mediator between Clusius and his Italian fellow scientists.

previous year that he was working on "*Fungis Commentariolo*" and that Aldrovandi was working on a similar subject.

"*Fungi exculenti*", i.e. edible mushrooms, are described first, in genera numbered I—XXI. From genus I we mention here *Morchella esculenta*, *Gyromitra esculenta* and *Morchella conica*.[39] (Tragus and Lonicerus mention the genus as "fungus porosus", Dodonaeus and Imperato as spongy mushroom, della Porta as "spongiolo" and G. Pona as "i Napoletani", while Gesner describes it under the name "early mushroom"). When speaking of genus II Clusius notes that he observed it on an island in Lake Balaton. Genus III corresponds to *Calocybe gambosa*. Three species are placed in genus IV. According to Clusius three species belong to genus V, but he describes, in fact, different specimens of *Polyporus squamosus*. Genus VI is *Pleurotus cornucopiae* (Dodonaeus describes it as "poplar mushroom", relying on Mattioli, who mentions that the mushroom can be seen on white poplar). Genus VII is a specimen of *Agaricus campestris*. In genus VIII three species are placed, one of which may correspond to *Lactarius piperata* (Tragus describes it as "fungus orbicolare", as does Lonicerus; Lobelius names it "forest mushroom"). From genus IX we may mention *Lactarius vellerus* and *Russula foetens* (Lobelius speaks of them as "*Fungi vulgatissimi*". Genus X includes two species, genus XI one species. Genus XII corresponds to *Cantharellus cibarius*) which in Tragus' system belongs to genus IV). In genus XIII three species can be identified as *Russula virescens* (by mentioning some characteristic features Clusius clearly separated this mushroom from the other *Russula* species) and *Russula adusta*. Genus XIV (mentioned by Gesner as "*Fungi genus magnum*", by Lobelius as "wood fungus", and by della Porta as "*suilli gulae novissima irritamenta o in napoletani silli*') and genus XV include two species each. In genus XVI Clusius lists three species, one of which can be identified, from the Hungarian name given by Clusius, as *Leccinum duriusculum* (in Tragus it is equivalent to genus VIII, in Lonicerus to genera VI and VII). It is in genus XVII that Clusius places *Amanita caesarea*, adding the mode of preparation as he saw it in Batthyány's court in 1584 (L. Anguillara[40] and Cesalpino mention it as egg-mushroom; Pliny described the young mushrooms as being covered by a volva; according to Glaucia the mushroom does the stomach good, while Galen records it as the only harmless mushroom). Genus XVIII is represented by *Macrolepiota procera* (Cesalpino mentions it as "scarogias ', and della Porta names it "conocchielle"). In genus XIX Clusius lists three species: *Ramaria flava*, *R. rufescens* and *R. botrytis* (della Porta reports on similar fungi rising from the stones of Monte Gargano). The three species described by Clusius in genus XX, can be identified as *Lactarius torminosus* (today no longer consumed) and *L. pallidus*. Genus XXI is represented by *Grifola frondosa*, which— according to Clusius — may reach considerable dimensions; the scientist could give no account of its taste as he never ate mushrooms, which led him to write: for this very reason Batthyány (who died two years after I moved to Frankfurt, i.e. in 1590, and whose memory I will cherish until my dying day) would be very amused if he knew that I am to write about (della porta mentions the mushroom as "gallinaccia").

In Clusius' system the edible mushrooms are followed by the poisonous fungi in genera I—XXVI. In the introduction he explains that the reason why the descriptions are shorter than those of the edible fungi is that he could not rely on antique authors (as they hardly dealt with "fungi perniciosi"), and the contemporary authors think it superfluous to deal with them; furthermore, his Hungarian friends did not know the names of all the poisonous mushrooms.

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[39] When discussing Clusius' mushrooms on the basis of Gy. Istvánffy's "Études et commentaires" (1900), mention is only made of species where the opinion has not changed; the species *Boletus* and *Russula* are presented according to the conception of G. Bohus.

[40] He was a pupil of Ghini in Pisa, and became the first warden of the botanical garden established in Padova in 1545; it was here that he instructed Aldrovandi and Calzolari. Later, in the service of the prince of Ferrara, he was director of the "Public gardens". The Italian botanist also made mycological observations.

Genus I is *Auricularia auricula* (according to Tragus it is on "older mushroom", because he saw it on the roots of older trees; it may be identical to Imperato's "fungo auricolare"; Lobelius described it as "Judas' ear mushroom"; Clusius mentions that dried mushroom in the pulverized state is used to cure inflamed throats). Genus II is *Pleurotus cornucopiae* (according to Tragus this fungus is very like the "plum mushroom" which he observed). Genus III is a *Coprinus* species (according to Cesalpino it is easy to mistake for the edible champignon). Genera IV and V are represented by *Grifola sulphurea*. Genus VI is a *Lactarius*, Genus VII includes three species: *Psathyrella candolleana*, *Amanita rubescens* and *Amanita vaginata*. Genus VIII refers to a white fungus growing on trees (Tragus calls it "fungus ad betulam"). Genus IX can be identified with *Russula foetens*. The identity of genus X is not clear. Genus XI is *Amanita vaginata*. In genus XII Clusius mentions five species, of which the fourth is *Amanita muscaria* (Clusius saw it sold in Frankfurt as a fly poison) and the fifth a *Cortinarius* (the fourth species is mentioned by Tragus, Cordus and Lonicerus as "fly-killing mushroom"). The two species of genus XIII are *Pluteus pellitus* and *Russula delica*. In genus XIV Clusius again mentions two species. The identity of the two species of genus XV is not clear. In genus XVI Clusius distinguishes three species, of which *Anellaria separata* and *Lepiota mastoides* can be mentioned here. Of the two species of genus XVII the second can be identified as *Paxillus involutus*. Genus XVIII includes a *Collybia* species. Mostly *Boletus* species are found among the eight species of genus XIX and according to Clusius these are very like the edible *Boletus* species: *Boletus erythropus*, which appears on several plates, *Xeroconmus subtomentosus*, *Leccinum aurantiacum*. In the author's opinion the third species given by Clusius is very like genus IV of the edible fungi and can be identified as *Tricholoma pardinum*. In genus XX four species are placed, of which *Boletus aureus* and *Leccinum crocipodium* are finely illustrated. Genus XXI is *Lactarius sanguifluus*. In genus XXII Clusius enumerates six species, of which *Hypholoma fasciculare* and *Collybia fusipes* are worth mentioning. Genus XXIII includes six species, the second of which is *Lactarius quietus;* the identity of the third is uncertain, the fourth species is *Clitopilus prunulus* (which is now widely consumed) and the fifth is *Ithyphallus impudicus* (according to Clusius it is like the mushroom described by H. Junius). The last species is "anonimo"; Clusius obtained it from Italy. He writes on the subject as follows: "when it is ripe the upper part opens up and the seeds which fill it become visible: they are similar in size to cyclamen seeds, . . . a friend of mine sent me some dry seeds from Italy, mistaking them for the seeds of some exotic plant, but I later identified them as mushroom".[41] Genus XXIV has three species. Genus XXV is *Hydnum coralloides*. Of the three species of Genus XXVI *Langermannia gigantea* is of interest (Tragus and Lonicerus mention it as "fungus ornatus", Durante and Colonna describe it under the name "vescia di lupo", Dodonaeus lists it as "fungus orbicolaris", and Lobelius as "a ball-shaped mushroom"; the first species of the genus is mentioned by Cesalpino as a fungus possibly identical with Pliny's "*Pezicae*", Imperato describes the second species of the genus under the name "fungo vescicario; in the case of the third species Clusius writes that the dried, pulverized mushroom is used to promote blood coagulation).

In the appendix ("*Appendicis Alterius Auctarium*") Clusius mentions two further fungi, and finally the "fungus coralloides cancellatus" sent him by N. di Calas in 1604 from the neighbourhood of Aix, which corresponds to *Clathrus cancellatus* (Gesner mentions the species by the name "fungus panis laceris similis igneus", and Cesalpino as "*fungus quem ignem silvestrem vocant*"). Clusius writes here about two further fungus species.

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[41] J. B. Barla's belief that Clusius was the first to find spores was probably based on this statement. In C. Roumeguère's opinion, however, it was P. A. Micheli, at the beginning of the 18th century, who discovered the spores and proved their existence by means of mushroom reproduction trials carried out in the Boboli Garden in Florence.

The effect of Clusius' micological activity

Clusius refers to Theophrastos, Dioscorides, Ateneo, Pliny, Nicander, Paulus Egineta, Galen, Avicenna, Mattioli and G. B. della Porta, whose works are used as sources in his monograph. In the descriptions he indicates where (mostly on trees) and when he found the fungi, cites from relevant works of other authors, gives the Hungarian, German, Italian and French names of the species, mentions the possible manner of utilization (as food, medicine, etc.) and describes the shape, colour, size and other specific features of the mushroom. No reference is made to the reproduction of fungi.

From Clusius' work we learn that some less valuable mushrooms were consumed in those days (e.g. *Polyporus squamosus*), while other valuable species were registered as poisonous mushrooms (e.g. *Boletus aureus*, *Leccinum auranticum*, *Lactarius sanguifluus* and those puffball species that Cesalpino knew in Italy as widely consumed mushrooms). The most important fungus species described by Clusius are: *Pleurotus cornucopiae*, *Polyporus squamosus*, *Grifola sulphurea*, *Russula foetens*, *R. adusta*, *Amanita muscaria*, *A. caesarea*, *A. vaginata*, *Lactarius pergamenus*, *Leccinum crocipodium*, *L. auranticum*, *Boletus edulis*, *Macrolepiota procera*, *Ramaria botrytis*, *Lactarius torminosus*, *Pholiota mutabilis*, *Collybia fusipes* and others.

Clusius dealt with fungi even after he had written his monograph (in 1604 N. di Calas sent Clusius some fungi, including *Clathrus cancellatus*, of which he gives an account to G. Pona, with whom he became acquinted in Verona); he describes *Clathrus ruber* in the fourth chapter of "*Exoticorum libri decem*" (Antwerp, 1605). In 1610 F. Imperato wrote in his letter to G. B. Faber: "it is not by seed that the fungi are reproduced, they grow out from decaying materials as discussed at length by our Carlo Clusius in the Exoticorum; to which I add that the cold steep of fungi when spread over the ground results in the rise of many fine edible mushrooms". In "*Curae Posteriores*" (1611) we find a contribution to Clusius' 21 edible fungus genera.

Clusius' mycological work was used by many authors besides the Bauhin brothers. Sterbeeck was the first to try to identify Clusius' species in his work "*Theatrum fungorum*" (1675), but he made a number of errors. He also took over Clusius' pictures and explanations (since Clusius' work remained hidden for a long time from historians of botany they declared Sterbeeck to be the founder of mycology, although in fact he only copied, interpreted and explained Clusius' monograph and Codex). J. Paius used Clusius as the only source in describing fungi in "*Methodus Plantarum*" (London, 1682) and "*Historia Plantarum*" (London, 1704). Clusius' identifications of fungi are frequently cited in the chapter on fungi in G. Turre's "*Historia Plantarum*" (Padova, 1586) and in S. Vaillant's work "*Botanicon Parisienne*" (Leyden—Amsterdam, 1727). J. B. Morandi explains some of Clusius' fungi in "*Historia Botanica*" (Milano, 1744). J. Barreliero used Clusius as a source in describing fungi in his book "*Plantae per Galliam, Hispaniam et Italiam*" (Paris, 1714). Besides Linné, P. A. Micheli identified many species described by Clusius in his work "*Nova Genera Plantarum*" (Florence, 1729). L. F. Marsigli collected a great many fungi while staying in Hungary as a military expert between 1669 and 1700. He systematized them and compared them with Clusius' species in "*Collectio fungorum vegetantium in regnis Croatiae et Hungariae*", and frequently cited Clusius in his work "*Dissertatio de Generatione fungorum*" (Rome, 1714). Among Clusius' interpreters we should mention E. Fries, Kickx (who did not, however, discover that Sterbeeck only copied Clusius' findings), M. Britzelmayr, Reichardt, and, of the Hungarian authors, K. Kalchbrenner, Gy. Istvánffy, J. Sadler and G. Bohus.

Mycologists have dedicated a number of species to Clusius, e.g. *Amphishaeria Clusiae* Pat., *Coccomyces Clusiae* (Lév.) Sacc., *Glonium Clusiae* (B. & C.) Sacc., *Phallus Clusianus* Reichardt, *Polyporus Clusianus* Britz., *Septogloeum Clusiae* Karst. & Har., some of which are already synonyms.

Finally, a quotation from C. Morren who summed up the essence of Clusius' life-work in the following words: with his works Clusius left an exact and full scientific history for the generations to come, which is based on the idea that it is the destiny of plants to live in community with human beings.

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A. Ubrizsy in Savoya

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THE EFFECT OF ARTIFICIAL LIGHT ON SOME PHYSIOLOGICAL AND PERFORMANCE PARAMETERS OF SOWS AND THEIR OFFSPRINGS

The keeping of domestic animals especially that of pigs has considerably changed during the last decade. The greatest part of pigs is kept in closed buildings under industrial management conditions. In these buildings light has an extremely important role in the keeping of breeding stock. In the industrial housing of pigs the question arises whether the sows producing fattened material should be kept in natural light conditions or it is sufficient to house them in windowless piggeries in artificial light. This was the main question of the series of experiments to which we wished to receive answer through performance, physiological, hygienic and breeding parmeters.

Because of the limited extent of the paper and the easier survey, the design of the method is summarized in Fig. 1. The basic material were the piglets of the experiments (n = 300) reared in an artificial, windowless farrowing house (group L_a) and the piglets (n = 300) reared in a naturally lit, traditionally built farrowing house (group L_n). The weaning of the basic material took place at an age of 40 days. The piglets remained in the farrowing house till their 60th day of age and after this time they were put into the piggery for breeding stock, namely 60 randomly selected animals from the traditional farrowing house and 60 gilts from the experimental basic material.

Group L_a and their offsprings (piglets and later fattened pigs) were always kept in an artificially lit environment whereas group L_n and their offsprings in farrowing houses with

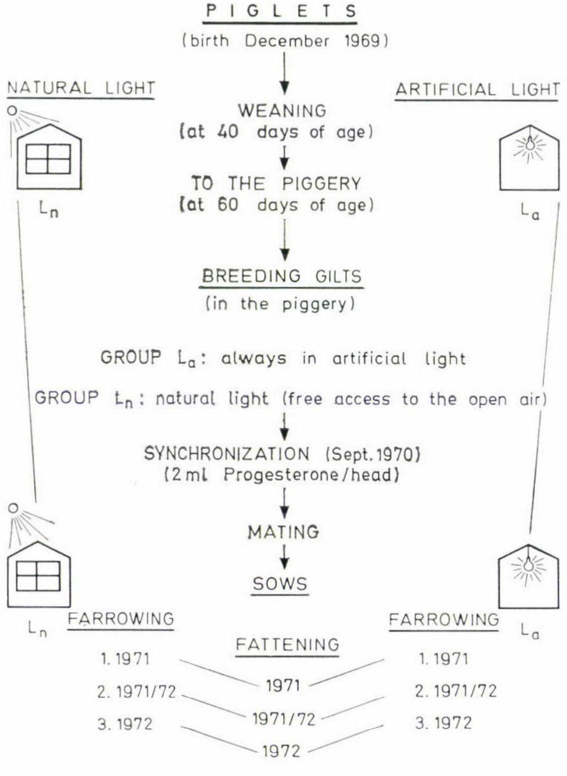

Fig. 1. Description of the method

windows. Special care was taken for the similar air change and microclimate of the piggeries which was controlled with suitable apparatus. The sows of this latter group were allowed to the open air.

The synchronization of the gilts took place in September 1970. For this purpose we used 2 ml Progesterone per head. The first farrowing took place in January 1971, the second in August 1971 and the third in February 1972. From the first farrowing 100—100, from the second 75—75 and from the third 50—50 piglets at an age of 68 days were taken out and fattened till a live weight of 105 kg. The nutrition of both groups was the same during the whole experiment.

Performance parameters of offsprings were as follows: 1. litter size at birth, at weaning (28 days of age) and before the transfer to the piggery for breeding stock (68 days of age); mortality, av. live weight of piglets at an age of 28 and 68 days; live weight gain between 28 (weaning) and 68 days of age; state of health. 2. av. daily live weight gain of fattened pigs, their food conversion and starch value conversion (kg food) starch value consumed (l per l kg gain), mortality, state of health; 3. the formation of the number of breeding sows, their fertility, conception rate, the interval between farrowings (days), state of health.

Physiological parameters were as follows: a) for the basic material (gilts): qualitative and quantitative blood picture (because of the limited space only the quantitative blood picture is demonstrated in Table 3); haemoglobin, total iron, serum Ca, serum anorganic P, lactic acid. b) for the piglets of the three farrowings (offsprings of the sows kept in artificially and naturally lit environments): qualitative and quantitative blood picture, blood sugar, total iron, haemoglobin, total protein, protein fractions.

In Table 1 we demonstrate the performance of piglets from which our basic material was taken as we have mentioned before. From the data of this Table it is evident that the piglets of group L_n after 40 days weanling age at an age of 60 days were 4.5 per cent heavier than those in group L_a. In the former the mortality was greater but the high litter size at birth must be taken into consideration in this case ($\bar{x}$: 11.3 piglets per litter). From these two farrowing houses 60—60 female piglets were chosen at random for using in the further experiments. From the second part of the method (Fig. 1) it is clear that group L_a was kept under artificial light as before, while group L_n in traditional piggery with windows. These animals had an access at any time to the open yard.

Table 1

Performance of piglets kept in artificial and natural light environment (basic material)

Environment	Litter size			$\bar{x}$ weight of a piglet			Live weight gain between weaning and 60 days of age kg	Mortality %
	at birth	at 40	60	1	40 weaning	60		
		days of age		days of age				
Artificial light (L_a)	10.7	9.75	9.6	1.35	9.82	14.09	12.74	10.5
Natural light (L_n)	11.3	9.7	9.6	1.30	9.72	14.72	13.42	15.4

Because of the shortening of farrowing periods the gilts were synchronized in September 1970 with 2 ml Progesterone per head.

In Table 2 the performance of the females kept in two different environments is summarized. According to these data the growth of group L_n was 3.7 per cent faster than that of group L_a. Concerning the number of mated individuals the L_a gilts were numerically superior to the L_n ones. This superiority can be attributed rather to chance than to the effects of the different light conditions.

Table 2

Performance parameters of breeding gilts (basic material)

Group	At an age of 60 days		Mated		Av. live weight gain from 60 days of age till taking into breeding (kg)	Excluded (n)	
	n	$\bar{x}$ kg	n	$\bar{x}$ kg		Constitutional deficiency	Disease
L_a	60	14.09	47	122.80	108.71	7	6
L_n	60	14.72	40	127.40	112.68	9	11

The haematological values of the experimental and control gilts are presented in Table 3. There were no statistical differences between these values. All were between the physiological limits. The number of red blood corpuscles was always bigger in group L_n whereas the number of white blood cells was bigger in the L_a gilts.

Table 3

Haematological parameters of breeding gilts (basic material)

Parameters		At 3 months of age		At 9 months of age	
		L_a	L_n	L_a	L_n
		groups		groups	
Red blood corp.	$\bar{x}$	4.8	6.1	5.6	6.2
(million/mm^3)	$s\pm$	0.27	0.76	0.64	0.63
White blood cells	$\bar{x}$	15.3	10.5	11.8	10.9
(1000/mm^3)	$s\pm$	4.38	2.06	1.89	1.72
Haemoglobin	$\bar{x}$	13.4	13.0	10.7	9.2
(g%)	$s\pm$	0.63	1.90	1.18	1.34
Total iron	$\bar{x}$	45.6	44.2	31.74	27.13
(mg%)	$s\pm$	—	—	3.62	3.97
Serum Ca (mg%)	$\bar{x}$	11.31	10.49	10.14	10.28
	$s\pm$	0.57	0.87	2.10	0.79
Serum anorg. P.	$\bar{x}$	5.54	5.10	4.73	5.58
(mg%)	$s\pm$	0.53	0.71	2.01	1.71
Lactic acid	$\bar{x}$	20.53	21.67	16.44	18.70
(mg%)	$s\pm$	3.26	4.80	8.73	7.83

The absolute haemoglobin quantity was below the normal level in both groups at an age of 9 months. This concerns the total iron level, too. The lactic acid level was not significantly greater in the gilts having a free access to the open air.

As we have already mentioned, the synchronization of the gilts was carried out at an age of 9 months and this was followed by the mating (Fig. 1).

The dates of farrowings, until the putting of piglets from the first, second and third farrowings to the fattening houses, are also presented in the same Figure. In every case piglets were weaned at an age of 28 days and remained in the farrowing houses till their 68th days of age. The fattened pigs were kept in the piggeries under the same environmental circumstances as before.

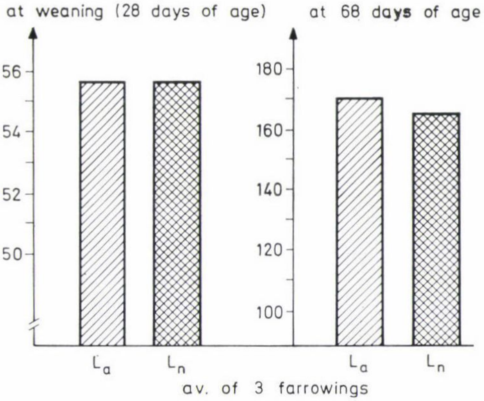

Fig. 2. Av. litter weight (offsprings of group L_a and L_n), (Mortality from birth to 68 days of age L_a: 18.2%, L_n: 18.8%)

The average weaning and 68 days weights of the piglets in all three farrowings are presented in Fig. 2. This Figure proves that in the first three farrowings at an age of 28 days (at weaning) there were no differences (55.7—55.7 kg) but at 68 days of age before being transferred to the fattening house the litter weight of group L_a was 3.3 per cent higher (170.9 kg) than that of group L_n (165.5 kg). The percentage of mortality was nearly the same (L_a = 18.2 per cent; L_n = 18.8 per cent).

Blood samples were taken from piglets selected at random from every group. The results of analyses are summarized in Table 4. There were no significant differences between the biochemical parameters. From the first farrowing 100, from the second 75 and from the third 50 animals were randomly chosen from every group for fattening. These pigs were kept under the same light environment as during their rearing period, that is to say in artificial and natural light. In the forming of groups the ratio of sexes was always the same, the initial live weight was about 20 kg, whereas the final live weight was 105 kg. The average performance (daily live weight gain, ADG), food starch value conversion of the three fattening periods are presented in Fig. 3. It can be concluded from this that the fattened pigs kept in two different light environments have reached nearly the same average daily live weight gain (L_a = 526 g; L_n = 528 g). There was likewise little difference between starch value consumption for 1 kg live weight gain (L_a = 2.96 kg; L_n = 2.91 kg) and for food consumption (1 kg live weight gain/4.19 kg L_a and 4.13 kg L_n). The number of breeding sows was also followed with attention in the experiments till their 3 years of age. During this time there were three farrowings. At the same time the number of sows still bred was taken into account on 1st November 1972.

According to the data presented in Fig. 4, 86.3 per cent of the sows kept under artificial light during their lives were fertilized whereas only 82.6 per cent of the L_n group. In the first farrowing 70.5 per cent of the L_a group and 75.0 per cent of the L_n group, in the second

Table 4

Haematological values of piglets. Av. of three farrowings (offsprings of groups L_a and L_n)

Date (age in days)		Group L_a			Group L_n		
		Red b. c. mill/mm^3	White b. c. 1000/mm^3	Haemoglobin g%	Red. b. c. mill/mm^3	White b. c. 1000/mm^3	Haemoglobin g%
7	$\bar{x}$	5.1	8.7	9.1	5.5	9.2	7.7
	s±	0.81	1.05	1.46	1.30	0.70	1.14
28	$\bar{x}$	6.5	11.3	10.1	6.7	10.3	9.7
	s±	0.39	2.14	1.05	0.38	0.45	1.11
68	$\bar{x}$	6.6	9.7	8.2	6.8	10.2	8.5
	s±	0.32	0.63	2.82	0.40	0.60	0.65

Total protein, gamma globuline, total iron and blood sugar values. Av. of three farrowings

Date (age in days)		Group L_a				Group L_n			
		Total prot. g%	Gamma glob. %	Total iron mg%	Blood sugar mg%	Total prot. g%	Gamma glob. %	Total iron mg%	Blood sugar mg%
7	x	5.53	24	30.8	90	5.33	26	26.3	100
	s±	0.78	—	4.87	13.02	2.20	—	3.72	13.67
28	$\bar{x}$	—	—	34.2	70	—	—	32.9	73
	s±	—	—	3.50	10.52	—	—	3.61	12.62
68	$\bar{x}$	—	—	27.5	70	—	—	28.7	67
	s±	—	—	3.69	10.41	—	—	3.05	3.88

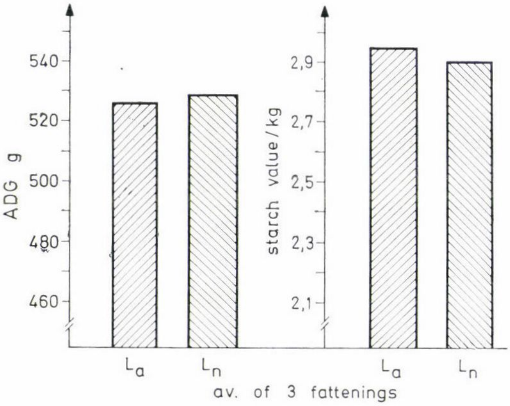

Fig. 3. Av. daily live weight gain of fattening pigs and starch value conversion (between 20 kg and 105 kg)

farrowing 45.3 per cent (L_a and 50.0 per cent (L_n), and in the third farrowing 30.5 per cent (L_a) and 32.8 per cent (L_n) of the sows took part.

After the three farrowings at the end of the experiment 18.9 per cent sows from group L_a and 22.5 per cent from group L_n were still in the breeding stock. Examining the nursing ability of the sows it can be stated that in the three experiments the total litter weight at weaning was in both groups nearly the same (L_a = 167.1 kg; L_n = 167.3 kg).

The reason of the exclusion from breeding was mainly the disease of extremities in the artificially lit group whereas in the group kept in traditional farrowing houses mastitis and the early decrease in milk production were the most frequent reasons for exclusion.

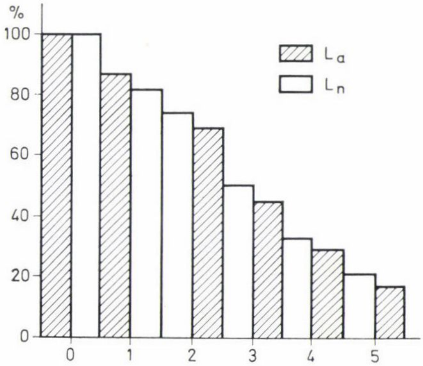

Fig. 4. The number of sows (0 = beginning of breeding, 1 = fertilized, 2 = farrowing I, 3 = farrowing II, 4 = farrowing III, 5 = breeding sows at 1st november 1972)

On the basis of the experimental results there seems to be no difficulty under farm conditions in keeping the Hungarian white meat breed sows producing fattened pigs and their fattening progenies in artificially lit farrowing houses during their whole life. It is evident that for drawing a complex conclusion it is necessary to take into account the cost conditions, too, which has not been our task in this work.

It must be emphasized once again that the aim of this series of experiments has been the examination of the influence of natural and artificial light on sows producing fattened pigs but not of sows producing gilts for breeding purposes.

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APPLICATION OF PATH ANALYSIS AND DISCRIMINANT FUNCTIONS FOR SELECTION IN BLACK GRAM (PHASEOLUS MUNGO L.)

Black gram (*Phaseolus mungo* L.) is an important pulse crop in India. It occupies the third position in acreage among the pulses in the country. However, little attention has been paid to various problems of its improvement with special reference to production breeding.

For a rational improvement in the yield, the understanding of correlations between the yield and its components and between the components themselves has been very useful. However, such estimates of correlations do not alone provide a comprehensive picture of the relative importance of the direct influences of each of the component characters towards this resultant trait. Path coefficient analysis, developed by WRIGHT (1921), serves this purpose effectively and more appropriately. Hence, this biometrical technique has been increasingly employed in measuring the extent and quanta of direct and indirect pathways to yield. Work along this line has been carried out by several workers, such as DEWEY—LU (1959) in crested wheat grass, BADWAL *et al.* (1970) in linseed, DABHOLKAR *et al.* (1970) in sorghum, SINGH—MEHNDIRATTA (1970) in cowpea, LAL—HAQUE (1971) and SENGUPTA—KATARIA (1971) in soybean, KUMAR—SAINI (1973) in rice, and others.

Besides resorting to path coefficient analysis, quantitative plant breeding calls for the building up of a suitable index in order to score out the superior genotypes, using some of the yield components. Towards this end the discriminant function is a very useful biometrical technique employed in working out different selection indices. This method, first developed by FISHER (1936), has been widely applied in several crops (SMITH 1936, SIMLOTE 1947 and SIKKA—JAIN 1958, in wheat; ROBINSON *et al.* 1951, in corn; ABRAHAM *et al.* 1954, in rice; PANSE—KHARGAONKAR 1949, in cotton; SWARUP—CHAUGALE 1962, in sorghum; SINGH—MEHNDIRATTA 1970, in cowpea; and others) as an aid to selection work.

The present paper deals with correlation, path coefficient and discriminant function studies in black gram involving different quantitative characters, including yield.

Sixteen varieties of black gram representing collections from different places in India were employed for the present investigation.

The experiment was conducted at the Agricultural Experimental Station of Calcutta University at Baruipur in the kharif season (June till about December) of 1973 using a randomized block design with four replications. Each variety in a replication was represented by a single row comprising 20 plants in each row. The distance between two rows was 50 cm, and 15—20 cm within each row. Pure line seeds were sown on 26th June 1973. All the cultural practices were carried out according to the usual method of black gram cultivation.

Five plants, excluding border plants, were randomly selected from each variety in each replication and observations were recorded on the following characters: plant height (x_1); number of pods per plant (x_2); number of branches per plant (x_3); days to flowering (x_4); length of the pod in cm (x_5); 100-seed-weight in gm (x_6); yield (plant in gm (x_7) and number of seeds per pod (x_8).

Statistical procedure. The mean values of five plants for each character were subjected to statistical calculations. For the estimation of genotypic and phenotypic correlations the formulae suggested by AL-JIBOURI *et al.* (1958) were employed.

Path coefficients were calculated following the procedure suggested by DEWEY—LU (1959) at genotypic level, and as applied subsequently by other workers (BADWAL *et al.* 1970, DABHOLKAR *et al.* 1970, KUMAR—SAINI 1973).

For the estimation of the discriminant function and selection indices required the method suggested by SMITH (1936) and developed by SIMLOTE (1947), SIKKA—JAIN (1958) and SINGH—MEHNDIRATTA (1970) was employed.

The mean performances of different varieties with respect to various plant characters are presented in Table 1. Analysis of variance for all the characters is given in Table 2. The latter table shows that all characters except the number of seeds/pod registered significant differences between the varieties under study.

Genotypic and phenotypic correlations between the yield and the yield-contributing characters and between these components themselves are given in Table 3. The seed yield showed positive correlations with pod length (x_5), number of pods (x_2), 100-seed-weight (x_6)

Table 1

Mean values of different characters in black gram

	Plant Reight (cm) x_1	No. of pods' plant x_2	No. of branches x_3	Days to flower x_4	Length of the pod (cm) x_5	100-seed-weight x_6	Yield/plant (gm) x_7	No. of seeds' pod x_8
T-27	254.12	77.00	3.00	88.50	4.39	2.66	11.94	6.45
T-9	85.13	44.50	3.00	41.00	4.21	2.79	8.45	6.47
B-76	86.26	55.50	3.50	38.70	4.21	2.67	10.94	6.42
KC-145	102.28	57.50	3.25	39.50	4.47	2.54	11.72	6.90
Mash-48	219.78	55.20	5.00	62.20	4.80	2.63	10.95	6.82
KC-184	94.90	50.20	3.25	40.50	4.28	2.50	7.54	6.75
IC-7545	104.99	37.50	2.25	38.00	4.32	2.45	13.10	6.50
L-64	203.41	47.20	3.50	57.70	4.54	2.75	9.30	6.67
IC-7574	108.34	43.20	2.50	38.00	4.36	2.85	8.90	6.57
Mash-1-1	209.32	50.00	4.50	60.70	4.38	2.09	8.50	6.87
UPU-2	193.93	37.70	3.75	74.70	4.37	3.04	8.19	6.52
L-35-5	212.41	47.50	3.00	68.50	4.05	2.59	7.51	6.17
UPU-1	101.82	50.70	3.25	41.70	4.23	3.09	10.85	6.67
NP-4	251.85	56.20	4.75	67.00	4.30	2.34	10.57	6.72
Set-I	222.71	52.20	5.00	61.00	4.33	2.75	8.71	6.50
Local	202.87	48.70	5.00	74.20	3.99	1.93	7.21	6.77

and number of primary branches (x_3), at both the phenotypic and genotypic levels, where as its relationship with the other two characters, viz. plant height and days to flower, was comparatively low and generally negative. Among the other component characters, the highest positive correlations at both levels were observed between plant height and days to flower. The number of primary branches also exhibited high positive correlations with plant height, days to flower and number of pods per plant, at both the phenotypic and genotypic levels. The length of the pod showed a positive correlation with the number of pods per plant only at the phenotypic level, while its relationship with the number of seeds per plant was positive at both levels. As regards the extent of relationship that the number of pods exhibited with other characters, it may be pointed out for the sake of clarity that at both the phenotypic and genotypic levels it showed positive correlations with plant height, number of primary branches, days to flower, seed yield and number of seeds per pod. As regards its relationship with the other two characters, viz. pod length and 100 seed weight, the phenotypic correlation values were positive while the genotypic ones were found to be negative, although low. The correlation values in other combinations between the component characters, however, were generally low and at times negative.

The computed path coefficients representing the contribution of the component characters to the yield are presented in Table 4 and diagrammatically represented in Fig. 1. The number of pods per plant, the length of the pod and the days to flower had high positive direct effects on the yield, whereas the height of the plant, the number of primary branches and the 100-seed-weight had negative direct effects.

Different discriminant functions obtained by taking various combinations of thre characters are presented in Table 5. The characters showing high positive direct effects in

Table 2

Analysis of variance for eight characters in black gram

Source	D. F.	Plant height x_1	No. of pods/plant x_2	No. of branches/plant x_3	Days to flower x_4	Length of pod x_5	100 seed weight x_6	Yield/plant x_7	No. of seeds/pod x_8
Block	3	1 851.34	298.50	1.56	14.86	0.076	0.079	10.40	0.03
Variety	15	16 531.81**	340.91**	4.52**	1 067.20**	0.140*	0.369*	12.95**	0.151
Error	45	624.75	115.25	0.84	6.65	0.035	0.108	4.03	0.10

* Significant at 5% level, ** significant at 1% level

Table 3

Phenotypic and genotypic correlations between eight characters

		Plant height x_1	No. of pods/plant x_2	No. of branches/plant x_3	Days to flower x_4	Length of the pod x_5	100 seed weight x_6	Yield/plant x_7	No. of seeds/pod x_8
x_1	P		0.2777	0.6898	0.8636	0.1348	−0.1981	−0.3934	0.0234
	G		0.4502	0.8353	0.9229	0.2058	−0.3663	0.0208	−0.0208
x_2	P			0.6732	0.2628	0.5720	0.0054	0.4660	0.0550
	G			0.7138	0.4975	−0.1530	−0.2913	0.2719	0.1707
x_3	P				0.5889	0.2179	−0.1311	0.2106	0.1505
	G				0.8603	0.1448	−0.5336	0.1595	0.3722
x_4	P					−0.0180	−0.5400	−0.1400	−0.0198
	G					−0.0087	−0.0949	−0.2432	−0.2592
x_5	P						0.2075	0.5334	0.5944
	G						0.2663	0.3409	0.2747
x_6	P							0.3182	−0.1317
	G							0.0579	−0.9059
x_7	P								0.0401
	G								0.1470
x_8	P								
	G								

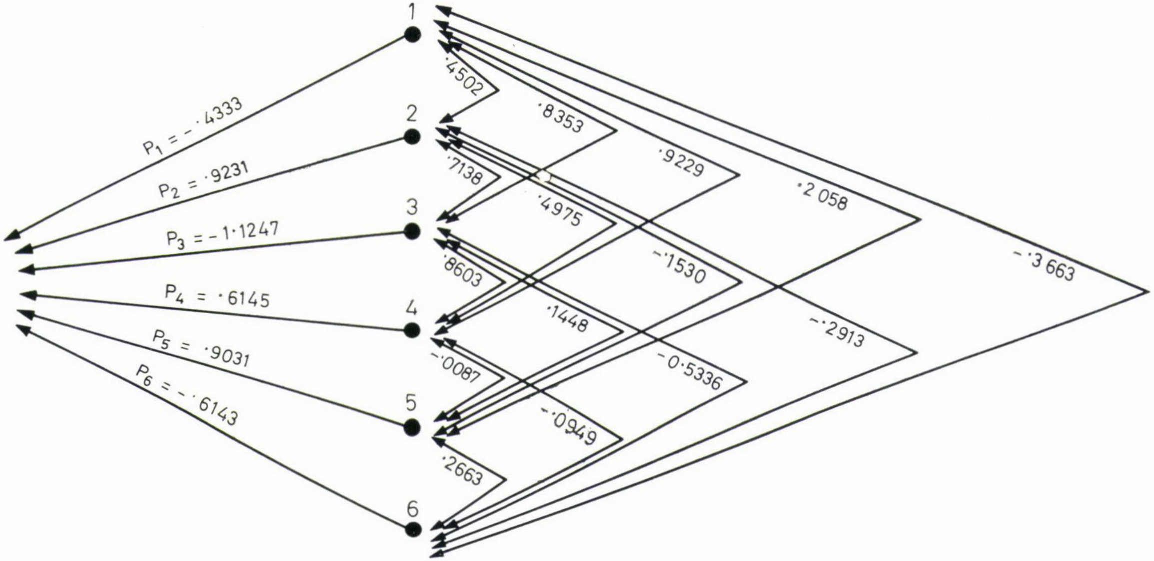

Fig. 1. A path diagram showing direct and indirect influences of different characters on yield. In the diagram, the single arrowed lines represent direct influence as measured by path coefficient (Pij) and the double arrowed lines indicate mutual association as measured by correlation coefficient (pij). (1. Plant height, 2. No. of pods/plant, 3. No. of primary branches/plant, 4. days to flower, 5. length of the pod, 6. 100-seed-weight, 7. yield/plant.)

Table 4

Path coefficient analysis of different characters on grain yield of black gram at genotypic level

	x_1	x_2	x_3	x_4	x_5	x_6	Correlation with yield (x_7)
Plant height (x_1)	*−0.4333*	0.4155	−0.9394	0.5671	0.1853	0.2250	0.0208
No. of pods (x_2)	−0.1950	*0.9231*	−0.8028	0.3057	−0.1381	0.1789	0.2719
No. of branches (x_3)	−0.3619	0.6589	*−1.1247*	0.5286	0.1308	0.3278	0.1595
Days to flower (x_4)	−0.3999	0.4592	0.9675	*0.6145*	−0.0078	0.0583	−0.2432
Length of the pod (x_5)	−0.0892	−0.1412	−0.1629	−0.0053	*0.9031*	−0.1636	0.3409
100 seed weight (x_6)	0.1587	−0.2688	0.6001	0.0583	0.2405	*−0.6143*	0.0578

path analysis have been used to build up the selection indices from these discriminant functions. While scoring the expected genetic advance it was found that the maximum value (125.645) was obtained by the function involving the yield and days to flower. The next important index included the yield, days to flower and number of pods per plant (96.161). The score values of the rest of the functions lagged far behind. With the help of the latter index the genotypic score values of different strains have been computed and presented in Table 6.

Various trends of association have been observed between the component characters themselves and between each of them individually and the yield. The yield was positively correlated with all the characters except days to flower. Pod length was positively associated with all the plant characters except days to flower (x_4), the highest value being observed in its relationship with the number of seeds/pod. This indicates that an increase in pod length

Table 5

Discriminant functions, genetic advance and relative efficiencies in black gram

Sl. No.	Selection indices	Expected genetic advance	Relative efficiency in (%) as compared to straight selection based on yield
1	$Y = 17\ x_7$	31.212	100.00
2	$Y = 1x_7 + 0.141\ x_2$	43.200	138.40
3	$Y = 1x_7 + 0.5003\ x_4$	125.645	402.55
4	$Y = 1x_7 + 0.8604\ x_5$	33.889	108.57
5	$Y = 1x_7 = 0.1393\ x_2 + 0.5604\ x_4$	96.161	308.09
6	$Y = -1x_7 + 0.5238\ x_2 + 0.0306\ x_5$	24.210	77.56
7	$Y = 0.0157\,x_7 + 0.0435\ x_2 - 0.0043\ x_4 - 1\ x_5$	15.364	49.22

x_1 = plant height, x_2 = No. of pods/plant, x_3 = No. of branches/plant, x_4 = days to flower, x_5 = length of pods, x_6 = 100-seed-weight, x_7 = yield/plant

Table 6

Genotypic score of 16 strains of black gram arranged in order of merit

Strain	Genotypic score	Strain	Genotypic score
T-27	72.27	L-64	48.21
NP-4	55.95	KC-145	41.87
Local	55.57	UPU-1	41.28
UPU-2	55.30	B-76	40.36
Mash-48	53.50	IC-7545	39.62
L-35-5	52.52	T-9	37.63
Set-^{1}	50.16	KC-184	37.23
Mash-1-1	49.49	IC-7574	36.22

Index used $1\ x_1 + 0.1393\ x_2 + 0.5604\ x_4$

would increase the number of seeds per pod. The 100-seed-weight had a positive association with the number of pods per plant but showed a negative correlation with the number of seeds per pod. Hence, an increase in the seed weight would be expected to effect a decrease in the number of seeds per pod. Among the characters showing negative correlations with the yield, the maximum value was registered by the height of the plant at the phenotypic level. This finding fits in very well with the present trend in plant type concept studies towards evolving high yielding varieties with short plant stature. The results of correlations obtained in the present investigation are in accordance with those obtained by SINGH—MALHOTRA (1970) in mung bean and BADWAL *et al.* (1970) in linseed.

While the above discussion on correlation pertains to the picture emanating at the phenotypic level, the path coefficient analysis has been based on correlation values obtained at the genotypic level. The number of pods per plant had the greatest positive direct effect on the yield. However, this direct effect was to some extent nullified by the negative indirect effects through plant height, number of branches and pod length, resulting in a comparatively smaller value for the total correlation. In the case of days to flower, it had a high positive direct effect on the yield but had negative indirect effects via plant height, number of branches and pod length. In the case of pod length, the direct effect was high and positive, but it was reduced to a lower value of total correlation through the indirect negative effects of other characters, viz. plant height, number of pods, number of primary branches, days to flower and 100-seed-weight. It is interesting to note that although the total genotypic correlations between the yield and three other characters, viz. number of primary branches, 100-seed-weight and plant height, were low and positive, the direct effects of the latter characters on the yield were negative. Thus, the total positive correlation between the yield and the number of branches was due to the indirect positive effects of number of pods per plant, days to flower, length of the pod and 100-seed-weight. Similarly, the positive correlation between the yield and the 100-seed-weight was due to the indirect positive effects through plant height, number of primary branches, days to flower, and length of the pod. The magnitude of the total positive correlation between the yield and the plant height was very low and it was manifested through the indirect effects of number of pods, days to flower, pod length and 100-seed-weight. Hence, in some situations dissimilarities appeared between the results of simple correlation and path coefficient analysis. A close examination of Table 4 and Fig. 1 revealed that almost all the yield components contributed to the yield through the number of pods per plant. It thus clearly indicates that the number of pods is the most important component of the seed yield. More or less identical results were obtained by SRIVASTAVA—SINGH (1971) in dwarf wheat.

In the discriminant function analysis the yield and three characters, viz. number of pods per plant, days to flower, and length of the pod, that had a positive direct effect on the yield were taken into consideration. The greatest value was obtained for the function involving the yield and days to flower. The next most important index includes the yield, days to flower and the number of pods per plant. Both these indices had a higher efficiency than straight selection on the basis of yield. In the present investigation, although the index including the yield and days to flower had the highest efficiency, it was considered justified to include the other important character, i.e. number of pods per plant, in the selection index when selecting the genotypes from the population. It is worth mentioning in this connection that the number of pods per plant showed the greatest positive direct effect as well as a positive correlation with the yield. Based on this index, compounded from the above three characters, the genotypic having the highest score may prove to be the most efficient in enhancing the present yield status of black gram.

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TYPES OF HOMOZYGOUS DIPLOID PRODUCTION FROM ANTHER CULTURE AND FROM POLLEN-DERIVED HAPLOIDS OF HIGHER PLANTS

Since the discovery made by GUHA—MAHESHWARI (1964, 1967) haploid forms of many cultivated and wild plant species have been produced from sterile anther and pollen cultures all over the world. It takes, however, many years of research work to produce haploid forms of further — not yet examined — species from anther culture and to find the optimum culture medium required for the induction.

The history, method and results of anther culture have been summarized by a number of authors (MAHESHWARI—RANGASWAMY 1965, NIIZEKI 1968, HESZKY 1971, SUNDERLAND 1971). During the past years researchers have disclosed the possible pathways for in vitro development of undifferentiated pollen grains and the course of haploid plant differentiation (GUHA—MAHESHWARI 1967, NAKATA—TANAKA 1968, NITSCH 1971a, 1972, HESZKY 1973a, RASHID—STREET 1973).

Haploid forms and the homozygotes produced from them are of special importance for breeding and genetic research work. Haploid research related with breeding began with the investigations of KATAYAMA (1934). He succeeded in obtaining several haploid plants by the X-ray treatment of *Triticum monococcum* pollen. On the basis of his results he evolved a "haploid method" for the utilization of haploid plants in breeding work (KATAYAMA 1950). However, no technique for inducing haploids existed which would make a wide application of the "haploid method" possible. Since the second half of the 1960's this possibility appears to have been created by the use of anther cultures.

The breeding and genetic importance of anther cultures as a method of haploid induction was emphasized by the work of MELCHERS—LABIB (1970) and KATAYAMA—TANAKA (1969), as well as by the lectures delivered on this range of subjects at a EUCARPIA meeting on "sterile culture method in plant breeding" held in Leeds in 1973 and at the international symposium on "haploids in higher plants" organized in Guelph in 1974.

It is, in fact, not the haploid plants but the homozygous diploid forms derived from them that give anther culture its practical importance. Thus, from the point of view of breeding and genetic research the anther culture includes the induction of haploid tissues, organs, embryos and plants on the one hand, and the production of homozygous diploid cells, tissues, organs and plants, on the other. In this paper we deal with the problems of the second range of subjects, and on the basis of our own experiences and of literary results try to outline the possible pathways for producing homozygotes in order to promote the wide application in plant breeding of anther culture as a method of inducing both haploids and homozygous diploids.

The possible ways of producing homozygous diploids are placed in four well distinguished groups (Fig. 1):

I. Production directly from the anther culture
II. Production from in vitro cultures of various haploid plant tissues
III. Production through physical and chemical treatment of haploid plants
IV. Production through spontaneous doubling of haploids.

Hereafter the theoretical and practical questions of methods belonging to the different groups are discussed in detail (Table 1).

Table 1

Different ways of homozygous plant production by haploid method

Types	Anther culture of diploid plant	Tissue culture of pollen haploid plant	Physical and chemical treatment of pollen haploid plant	Spontaneous doubling of pollen haploid plant
	I	II	III	IV
Possibility	Endoduplication during pollen development in vitro	Somatic mosaicism of haploid plant Endomitosis of callus cells	Effect on mitosis or meiosis	Spontaneous doubling of vegetative or generative cells
Method	Anther culture of immature anthers	Tissue and organ culture of leaf leaf midveins stem stem pith other somatic tissues	Colchicine treatment of plantlet vegetative bud axillary bud leaf axils other parts of plant	Production and cultivation of large number of pollen plants

I. *Production of homozygous plants directly from the anther culture (Fig. 1/I)*

In the anther culture not only haploid plants can be raised from the pollen grain; this fact was called attention to by the results of Nishi—Mitshouka (1969) and others. Plants of different ploidy levels (x, 2x, 3x, 5x) were raised from the anther culture of *Oryza sativa* L.

The phenomenon was explained only a couple of years later on the basis of results of investigations made with anther cultures by Narayanaswamy—Chandy (1971) with *Datura metel* L., Chandy—Narayanaswamy (1971), Narayanaswamy—George (1972) with *Atropa belladonna* L., Engvild—Laursen—Lundqist (1972) with *Datura innoxia* Mill., Raquin—Pilet (1972) with *Petunia* sp. and Engvild (1973) with *Petunia axillaris*. According to the general opinion now current, diploid reconstitution (rediploidization) is caused by the endoduplication of the haploid pollen grain prior to embryogenesis. The induction of embryogenesis, according to Norreel (1973), "is just before the first pollenic haploid mitosis, during the haploid mitosis or after this division". Endoduplication must thus take place immediately before or after the first pollenic haploid mitosis. According to Engvild—Laursen—Lundqvist (1972) it has two variations:

a) a diploid plantlet from an older uninucleate microspore in the stage of DNA replication prior to the first pollen mitosis;

b) a diploid plantlet after fusion of the two nuclei in a binucleate microspore after the first pollen mitosis.

It follows from the above that owing to an endoduplication occurring during the in vitro development of the pollen grain, diploid but already homozygous pollen grains are produced in the anther, and some of the plants raised from these anthers following the embryoid organization will also be homozygous diploids.

It is, however, necessary to note that, according to Narayanaswamy—Chandy (1971) "fusion between the two germ cells with the tube nucleus (vegetative nucleus) may take place in pollen grain to form a triploid cell, functional in embryogenesis, thereby giving a triploid plant". However, the formation of plants of any ploidy level other than diploid is not dealt with in our paper.

In the case when callus tissue is formed from the pollen grains in the first step instead of the differentiation of embryoids (Fig. 1/I—B), it is theoretically possible that, owing to the above described endoduplication and due to the endomitosis of callus cells, the cells of the haploid callus tissue induced in the anther culture will be partly diploid. Some of such plants raised from callus tissue may thus be homozygous diploids.

It deserves attention, however, that diploid plants may arise from anther cultures in a different way too. It has been reported about a number of plant species that the embryoids or callus tissue differentiating in the anther culture originate from the connective tissue of the anther and not from the pollen grains (Konar—Nataraja 1965). The plants will be diploids in this case, too, though not homozygous; they remain heterozygous! Special attention should therefore be paid to the origin and source of the diploid plants raised.

To sum it all up, we can state that homozygous diploid plants can be raised directly from anther culture; this possibility is offered by various ways of endoduplication. This technique may be regarded as the simplest and shortest way of producing homozygotes. It has the disadvantage that the endoduplication of pollen grains in anther culture is a process whose artificial control is difficult, and the number of diploids obtained is higly varying and generally low.

II. *Production of homozygous plants from in vitro cultures of various tissues or organs of haploid plants (Fig. 1/II)*

According to the cytological examinations of plants raised from pollen, the somatic tissues of haploid plants include haploid, diploid and aneuploid cells (Collins—Legg—Kasperbauer 1972). The somatic variety of haploid plants is characteristic of the tissue cultures prepared from them, too. This statement is confirmed by the results of cytological examinations performed by Maliga—Szilágyi (1973) in the species *Nicotiana tabacum* L. In tissue cultures prepared from haploid plants diploid cells may also occur — besides those mentioned before — through the endomitosis (Nitsch 1971b) or fusion (Niizeki—Oono 1971) of haploid cells.

The higher rate proliferation of haploid cells in the haploid callus tissue ensures the rapid growth of the proportion of diploid cells during incubation, and — at the same time — makes it possible to raise a relatively large number of homozygous diploid plants from the cultures. On the basis of these facts Kochar—Sabharwal—Engelberg (1971) and Nitsch (1971b) developed a tissue culture method to produce homozygotes.

The method is the following (Fig. 1/IIA-B): the vegetative parts of haploid plants raised from anther culture (stem, leaf, etc.) are isolated on a sterile culture medium where the tissues become callose. From the undifferentiated callus plant regeneration is induced. According to the results numerous diploid plants can be raised from the cultures. The method has, however, the disadvantage that the production of diploid plants takes a relatively long time (haploid induction, raising of haploids, haploid tissue isolation, callus induction, etc.). Kasperbauer—Collins (1972) therefore elaborated a quicker method described as follows: "The haploids were verified cytologically soon after emergence from the cultured anthers. They were screened under field or greenhouse conditions. Leaf midveins from selected haploid plants were trimmed of lamina and cultured on a chemically defined medium. Leafy shoots emerged in about 3 weeks. Plants regenerated from aged leaves (those that remained on the plant 3 to 4 weeks after attaining full expansion) included one-third diploids and two-thirds haploids".

According to the authors "the whole process is rapid enough that the selected haploids, their reconstituted diploids and the seed-borne progeny of the reconstituted diploids can all

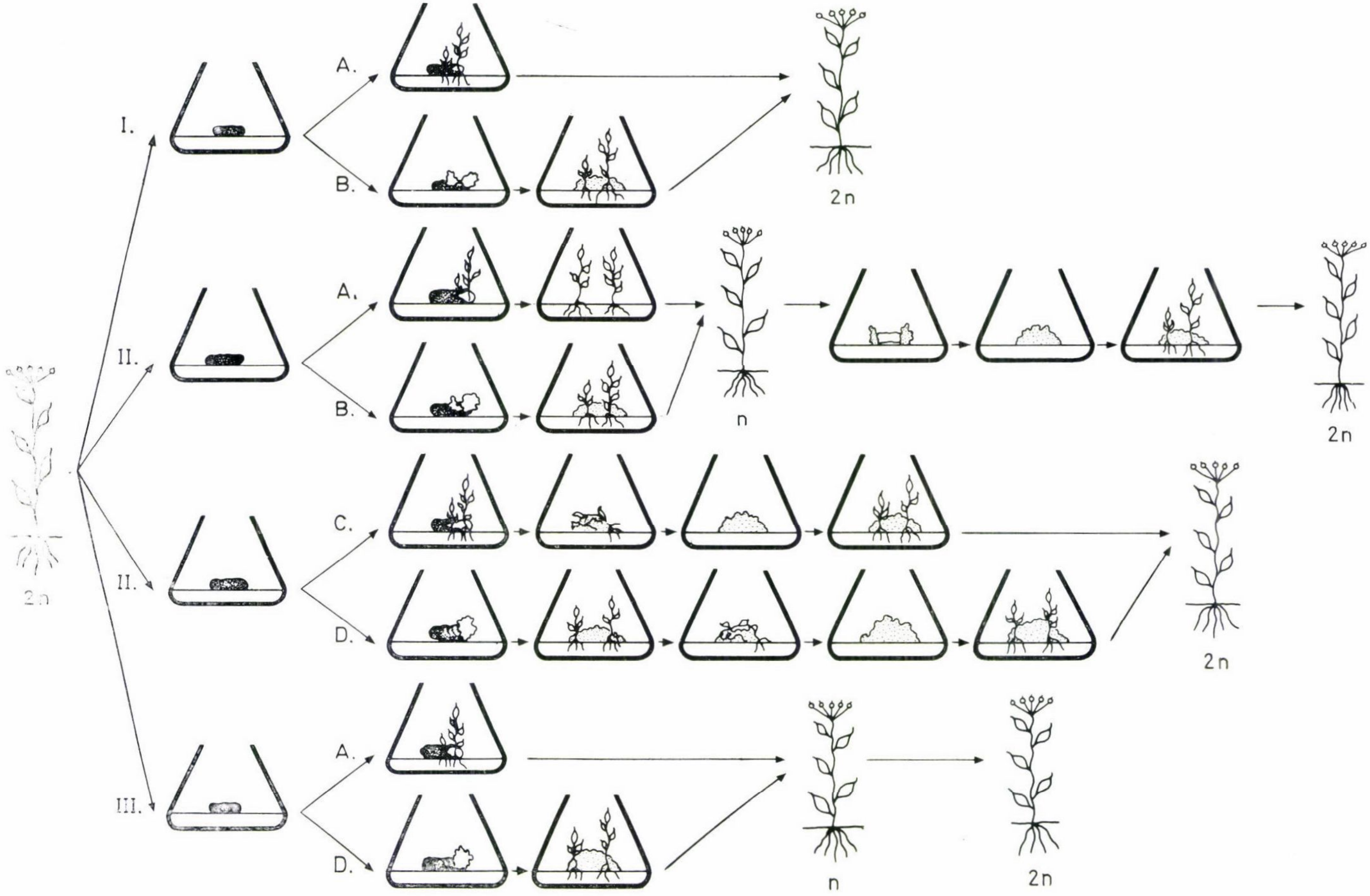

Fig. 1. Types of homozygous diploid production from anther culture and pollen-derived haploids of higher plants: I. production directly from anther culture; II production from in vitro cultures of various tissues of haploid plants; III. production through physical and chemical treatment or spontaneous doubling of haploid plants

be taken to the same field plot for testing in less than 18 months after the programme is initiated".

In spite of its excellence and the expectation that it will be widely introduced, the method has a drawback, namely, that the in vitro technique is discontinued and raising the haploids in the greenhouse or in the field takes a relatively long time. Starting from these two factors HESZKY (1973b) evolved a complete in vitro technique (Fig. 1/II C–D), the main point of which is that both the haploid and the homozygous diploid induction take place in the course of an unbroken laboratory culturing work. It is the anthers of heterozygous plants that enter and homozygous plants that leave the laboratory. The method shown in Fig. 1/II C–D is, in short, the following: The anthers of heterozygous plants are isolated on a culture medium. From plantlets growing in the anther culture, sterile root tips are taken. Plantlets which appear to be haploid in mitosis are placed on a culture medium where they become callose. The somatic variety of haploid plants guarantees that a part of the plants raised from the induced callus tissue will be diploid. Plantlets found to be diploid during the cytological examination of plantlets raised from the callus can be delivered to the breeders. From the haploid plants callus can be repeatedly induced, so the work will be unbroken: homozygous diploid plants can be continuously produced and — at the same time — the haploid forms maintained.

It can, in fact, be established that, owing to the somatic variety and endomitosis, numerous diploid cells can be found in vitro cultures prepared from various tissues or organs of haploid plants. The higher rate of proliferation of diploid cells in cultures ensures that homozygous diploid plants can be raised in large numbers even after a relatively short time of incubation. At the same time, the cultures provide the possibility of maintaining the haploid forms too, which is a very important aspect of both breeding and genetics.

III. *Production of homozygous plants through physical and chemical treatment of haploid plants* (Fig. 1/III)

Of various radiation treatments and chemicals acting on mitosis and inducing polyploidy, colchicine has at present the widest application in the case of haploid plants. By colchicine treatment many homozygous and autopolyploid plants have been produced. TANAKA—NAKATA (1969) and NIIZEKI—OONO (1971) ensured rediploidization in haploid plants raised from anther culture by colchicine treatment (Fig. 1/III A–B).

The researchers have applied the following forms of colchicine treatment:

1. colchicine treatment of plantlets,
2. colchicine treatment of axillary buds or leaf axils.

The methods of colchicine treatment are briefly summed up below on the basis of results obtained by BURK—GWYNN—CHAPLIN (1972):

Colchicine treatment of plantlets: When approximately 1 cm in length, the emerging plantlets from anthers were removed and placed in a porous crucible that was immersed in 0.4 per cent aqueous colchicine for 3 or 4 hours. The plantlets were rinsed by removing the crucible from the colchicine, allowing it to drain, and placing it in water. Thereafter the plantlets were planted in soil.

Colchicine treatment of axillary buds: The haploid plantlets were grown to the flowering stage. After flowering the plants were topped and axillary buds allowed to grow. Several axillary shoots from each plant were rooted and allowed to grow to a height of 30 or more centimeters before being cut back to three nodes. The terminal bud was covered with a paste of tragacanth gum containing 0.4 per cent colchicine (or 0.4 per cent lanoline) (TANAKA—NAKATA 1969), and the preparation kept moist for three days.

With the above treatments the authors were able to raise diploid plants in an average of 31.7 per cent of the cases. As opposed to the methods described earlier the authors hope that "the speed and simplicity of the method qualifies it for use in a large scale breeding program".

The colchicine treatment method is thus suitable for the production of diploid plants. As to its efficiency, it is more or less equal to that of the methods mentioned earlier. It is a simple method, its only disadvantage being that it cannot be used with all plant species.

IV. *Production of homozygous plants through spontaneous doubling of haploid plants* (Fig. 1/III)

The frequency of spontaneous doubling is generally low. About 1% of *Nicotiana tabacum* plants examined by Sunderland (1971, 1973) gave rise to mixed inflorescences and about 2% of those examined by Kasperbauer—Collins (1972). According to Niizeki—Oono (1971) and Heszky—Pauk (1975), haploid rice plants were completely sterile, but occasionally fertile grains developed as a chimera on haploid ears and from these seeds normal diploid plants grew.

Nevertheless, in cases where large numbers of pollen plants can easily be produced, a low frequency such as this will soon provide a working number of pure lines (Sunderland 1973).

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CHARACTERISTICS OF GROWTH AND DEVELOPMENT IN SHEEP

I. DEVELOPMENT AND SEXUAL MATURITY OF LAMBS

In disclosing the characteristics of growth and development Csirvinszkij and Hammond rendered great services. They were the ones to call attention to the fact that the development of young animals was divided into phases separated by more or less perceptible qualitative changes. Under the influence of genetic factors like breed and individuality the successive phases of growth and development may be of different duration, but they may also be influenced to a considerable extent by the effects of external factors like feeding and training.

The breeder's work will only be successful if he satisfies the constantly changing needs of animals during their development in such a way as to enable them to produce the utmost of their biological capacities when used for breeding.

In the classic, capitalist-structure farm, large-scale sheep-breeding was decentralized. If possible, each unit of the farm should have a flock. Sheep-farming was thus based on sheep pastures and on the utilization of agricultural by-products; the profit depended on economic maintenance and the skill and conscientiousness of the shepherd.

On large-scale farms the production forces have made rapid progress over the last decades. In certain branches of stock-breeding — primarily in poultry farming — the so-called industrial form of keeping has gained ground rapidly, but this is regarded as the really up-to-date operative structure in other branches of stock-breeding too. Its preconditions are:

- concentration of the stock,
- areal integration,
- rapid increase of output.

All this cannot be realized without the practical application of scientific results attained in the fields of biology, genetics and feeding. In industrial-scale production economic efficiency is an aim that cannot be achieved within the traditional organizational framework, but requires complete reorganization.

Increased fertility is the precondition for the widespread introduction of hybridization in sheep-breeding as the meat production will increase primarily through a higher rate of multiplication (VERESS 1974).

Modernization in sheep-farming makes it opportune to survey the currently prevailing system of raising lambs.

The first phase of raising lambs, which is far from unimportant, is the embryonic life. If the mother is insufficiently fed, the newborn lamb will have

- a lower birth weight,
- reduced resistance and viability,
- a smaller number of secondary follicles composing the fleece which through the thickness of the fleece later has an effect on wool production too.

As long as one or, at most, two lambs were expected to be born at a time, no special attention had to be paid to keeping and feeding during the gestation period. Today we know that overabundant feeding during the gestation period — especially to animals in first lamb — may inhibit the development of the udder glands, and the over-large embryo causes difficulty in lambing. An insufficient phosphorus supply, or A and E hypovitaminosis, decrease the viability of the lamb. Selenium deficiency, which occurs in many places in Hungary — when coupled with an insufficient vitamin E supply — may cause degeneration of the skeletal muscles in newborn lambs.

In a flock selected for high fertility, OWEN (1969) observed that when the optimum energy level required for 3—4 embryos was not ensured, abortion occured due to ketosis in the last phase of pregnancy, sometimes also causing the ewe to perish.

In 1966 in the combing merino stock of the Hortobágy State Farm the average weight of young rams born at single births was 4.2 kg and that of ewe lambs 4.03 kg.

In the carefully prepared stock, young rams born at twin births were lighter by only 8 per cent, and ewe lambs by 7 per cent than those of the same sex born at single births.

According to the investigations of KOVNEREV (1973) twin lambs are smaller by 20—21.5, triplets by 31—35, quadruplets by 41—47 and quintuplets by 50 per cent compared to those originating from single births. As a matter of interest he mentions that in litters of mixed sex the young rams are always heavier at the expense of the ewe lambs.

In the Romanov and Finnish stocks on our experimental farm, the differences in birth weights between smaller and larger litters were far less pronounced than those published by Kovnerev. In fertile breeds the live weights of newborn lambs are undoubtedly lower, but survival is determined first of all by the live weight at birth rather than by the size of the litter (Table 1).

Table 1

Relationship between the birth weight of lambs and their survival until the age of 28 days

	Romanov			Finnish landrace		
	born	survived	perished	born	survived	perished
	n = 166	n = 141	n = 25	n = 74	n = 53	n = 21
Index	100	84.9	15.1	100	71.6	28.4
Brith weight kg						
$\overline{x}$	2.38	2.54	1.50	1.92	2.42	1.47
s	0.87	0.82	0.58	0.60	0.54	0.46
cv%	32.3	32.3	38.7	31.3	25.6	31.7
P		< 0.1%			< 0.1%	

In the Romanov and Finnish landraces the birth weights of surviving lambs were 2.54 and 2.42 kg, while the average weights of the ones that perished were 1.50 and 1.47 kg.

Special attention should be paid to the tending and feeding of lambs born with lower weights, particularly in the first 5—6 months; this period should be regarded as a prophylactic phase similar to that in artificial calf-breeding. The growth of lambs in the first three weeks as a function of milk consumption — if the milk contains 7.5 per cent butterfat — is given below.

Daily weight gain	Amount of milk required a day
g	litre
150	0.950
200	1.150
250	1.350
300	1.600
350	1.800
400	2.000

In the merino stock of the Hortobágy State Farm, with the collaboration of Imre Kenyeres, we studied the weight gain of lambs during the lactation period until the age of 70 days. Although the Hungarian merino is one of the lighter-weight European sheep breeds, the weight gain of lambs in the first 30 days proved to be surprisingly high (Table 2). After 30 days the decreasing milk production of the ewes was less and less able to supply the increasing energy requirement of the lambs. Thus, the more lambs there are born, the less able the milk of the ewes will be to supply the optimum energy requirement of the lambs in this phase of development. Therefore, if three or more lambs per lambing are reckoned with, some method must be found of weaning at the age of 1—2 days and artifically rearing, either for all the lambs or for all but two.

According to CHARLET-LERY *et al.* (1954) lambs can be weaned at the age of 21—32 days if the proper composition and energy level of feeding is provided for.

Table 2

Development of lactated combing merino lambs in the first 75 days of life

Indices	Young rams		Ewe lambs	
	single	twin	single	twin
Number of lambs	164	52	170	50
Average birth weight, kg	4.20	3.89	4.03	3.76
Daily weight gain:				
in the first 30 days, g	280	274	273	262
between 30 and 75 days of age, g	190	196	195	202
from birth to the age of 75 days, g	227	227	226	226
average weight at the age of 30 days, kg	12.65	12.10	12.22	11.63
average weight at the age of 75 days, kg	21.20	20.90	21.00	20.75

Between 1968 and 1973 more than 2000 lambs were weaned at different ages and fed on feed mixtures of different composition. The conclusions concerning the development of young lambs are summed up as follows:

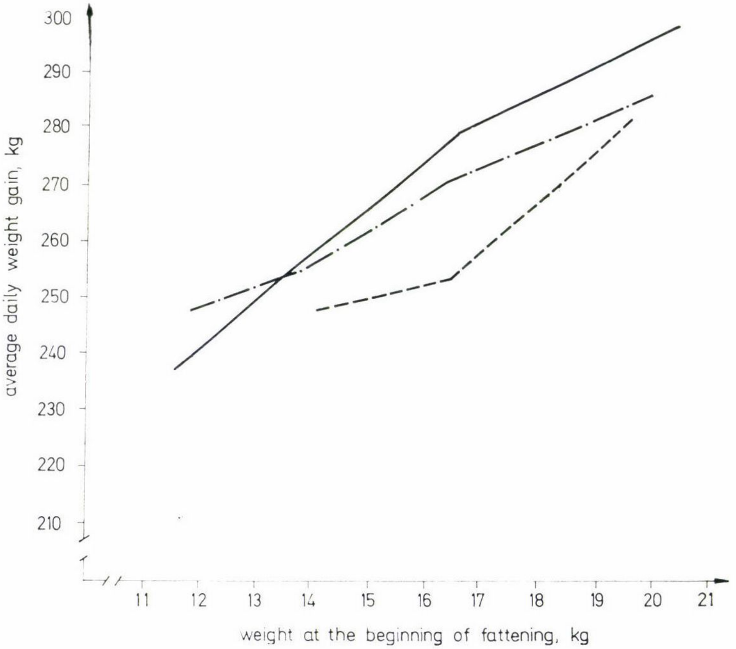

Fig. 1. Trend of daily weight gain during fattening as a function of the season and initial weight in young merino rams (———— I. winter 12.11—31.1.; — — — — II. summer 2.4.—25.6.; —.—.— III. autumn 18.8—29.10.)

Lambs having attained a weight 2.5—3 times their birth weight can be weaned at the age of 30 days or so and fed on a feed mixture of proper composition without any harm;

- if weaning takes place later, the weight of the lambs at weaning depends on the milk production of the ewes to a considerably higher degree, and reflects the genetically determined growth capacity of the lambs to a lower extent;
- differences in live weight between lambs weaned at the same age but with different weights are maintained during the 60—70 day period of fattening following weaning. The daily weight gain of lambs weaned with higher weights is significantly greater than that of lambs weaned when lighter (Figs 1—2). The only exception is the result of a summer fattening of ewe lambs in Fig. 2. In the case of ewe lambs put to fattening when older — and therefore heavier — the hot summer was not favourable for gaining weight.

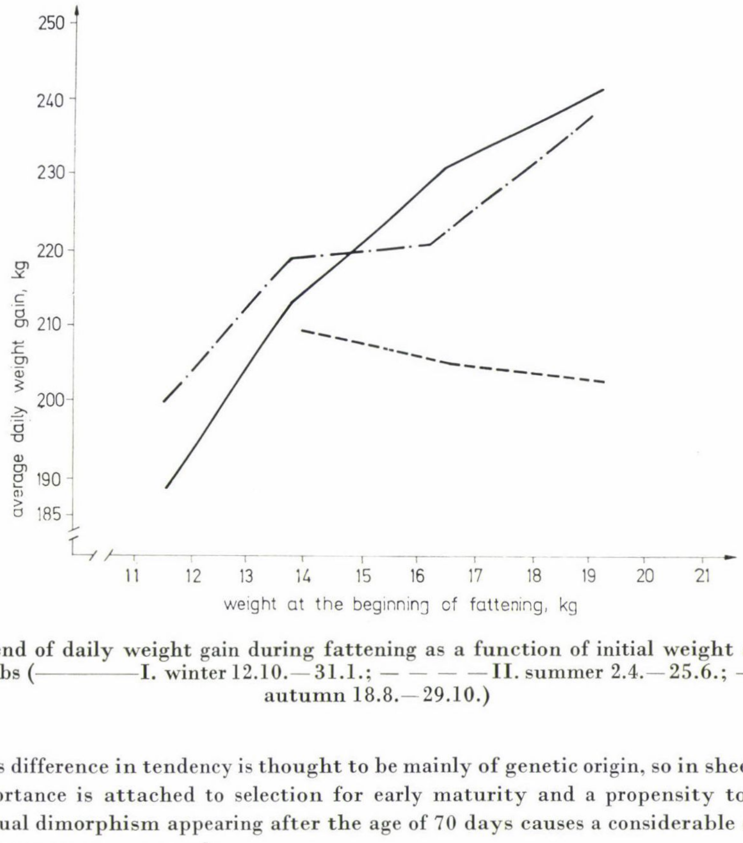

Fig. 2. Trend of daily weight gain during fattening as a function of initial weight and season in ewe lambs (————I. winter 12.10.—31.1.; — — — —II. summer 2.4.—25.6.; —.—.—III. autumn 18.8.—29.10.)

This difference in tendency is thought to be mainly of genetic origin, so in sheep breeding great importance is attached to selection for early maturity and a propensity to fattening.

Sexual dimorphism appearing after the age of 70 days causes a considerable differentiation of the two sexes as regards

- daily feed consumption,
- daily weight gain, and
- growth capacity.

Combing merino lambs weaned at 6 weeks old and separated by sex were fattened in groups of 20 for 72 days in six replications. The granulated feed mixtures fed ad libitum contained 64 per cent starch equivalent and 15 per cent crude protein. A significant difference in

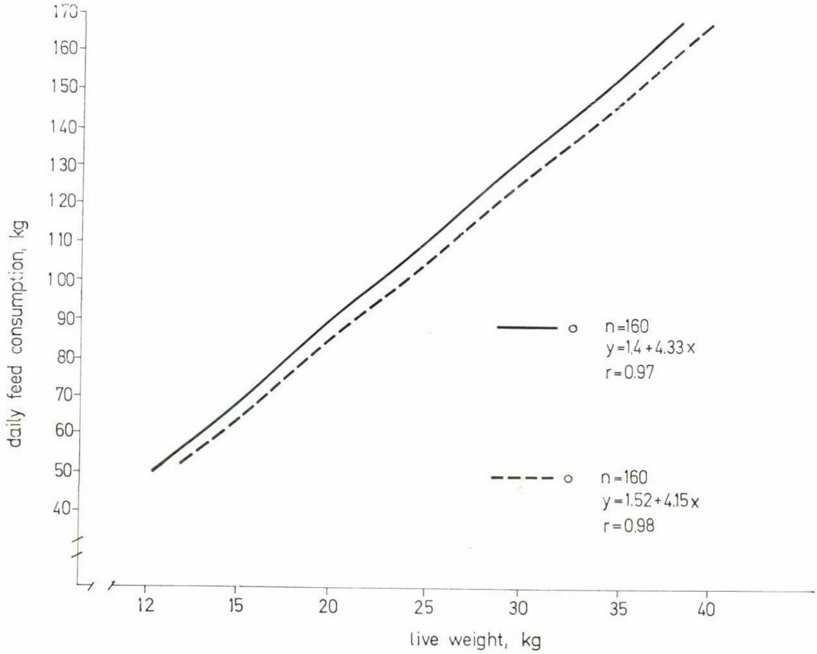

Fig. 3. Relationship between live weight and daily feed consumption during fattening in combing merino lambs weaned at the age of 6 weeks (during the 72 days from 18.8.—29.1.)

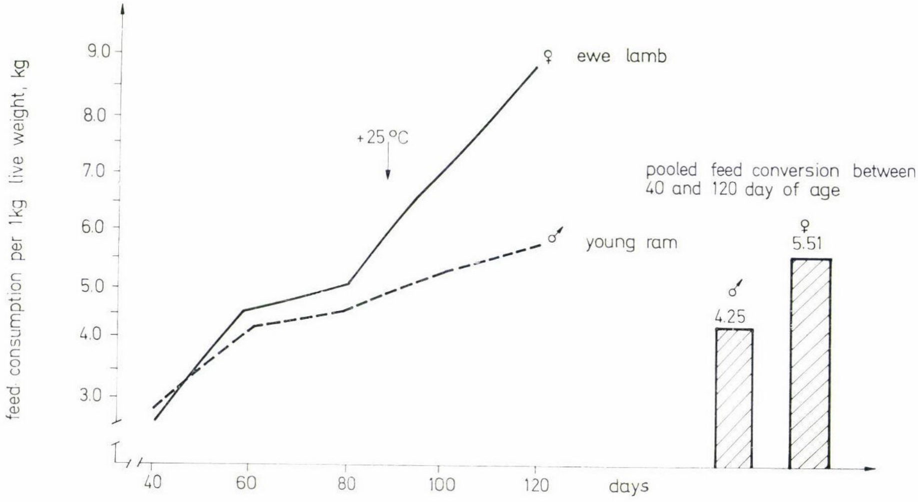

Fig. 4. Periodic and total feed conversion of fattening lambs in the course of summer fattening (2.4.—24.6.)

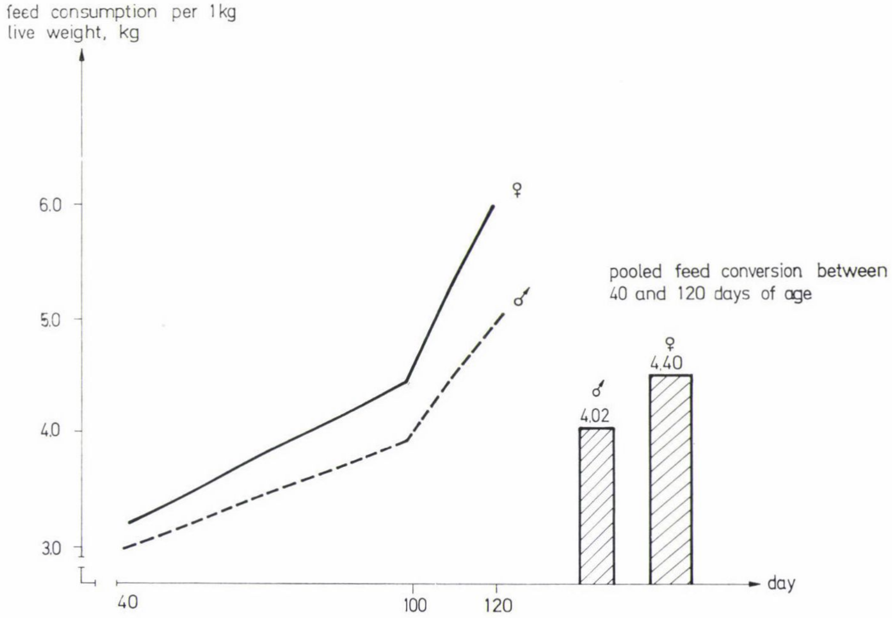

Fig. 5. Periodic and total feed conversion of fattening lambs in the course of autumn fattening (18.8.—29.10.)

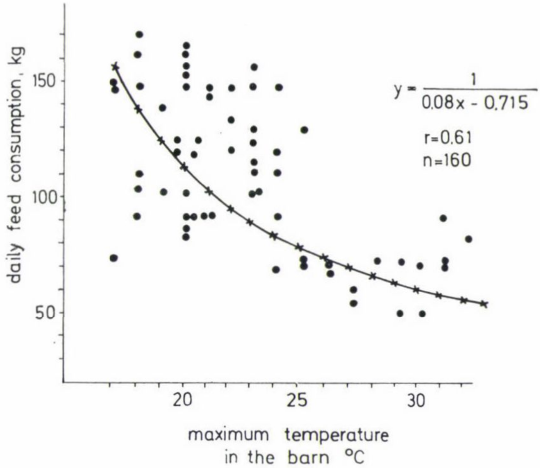

Fig. 6. Relationship between the daily temperature of the sheep-barn and feed consumption by young combing merino rams

feed consumption was found between the sexes. Young rams consumed some 4 per cent (2.56 per cent in starch equivalent), ewe lambs 4.5 per cent (2.88 per cent in starch equivalent) of their respective body weights a day (Fig. 3).

We find the explanation of this difference in the greater stress facing the ewe lambs later in their lives during pregnancy and lactation. Therefore, in a certain phase of development

— even before sexual maturity — the digestive tract of the ewe lambs develops at a faster rate than that of the rams.

It thus follows that

- from the age of 70—75 days the ewe lambs require a larger quantity of dry-matter than young rams of the same weight;
- the ewe lambs complete the intensive phase of muscle development sooner and therefore begin to form their fatty tissues earlier and more intensively.

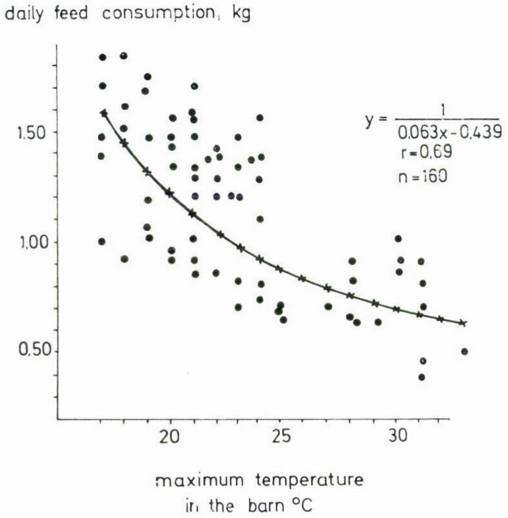

Fig. 7. Relationship between the daily temperature of the sheep-barn and feed consumption by combing merino ewe lambs

Lambs of both sexes arrive at a stage of development when the rapidly increasing feed consumption is not followed by the same rate of daily weight gain; this indicates the end of the intensive phase of muscle development.

It is at this point that feed consumption should be restricted, otherwise luxury consumption has to be reckoned with. According to our investigations, in the case of early weaning and rapid fattening this stage is reached in the combing merino breed at the age of about 100 days in ewe lambs, and 140 days in young rams (Figs 4—5).

In warm weather when the daily maximum temperature of the sheep-barn exceeds 25° C the daily feed consumption, which was previously highly variable, considerably decreases, as is clearly shown in Figs 6 and 7. Such a decrease caused by the heat is particularly noticeable in the feed uptake of ewe lambs. The differences found between the groups suggest at the same time that the different individual heat tolerance of the animals offers the possibility of a successful selection in this field too.

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ELECTROPHORETIC ISOENZYME STUDIES ON THE *AEGILOPS OVATA* × *TRITICUM TURGIDUM* SSP. *CARTHLICUM* AMPHIPLOID

The amphiploid *Aegilops ovata* × *Triticum turgidum* ssp. *carthlicum* was produced by treating the F_1 hybrid grains with 0.05 per cent colchicine and 10 ppm gibberellic acid (Fig. 1.). In order to obtain more information about the amphiploid, the electrophoretic patterns of esterase (E.C.3.1.) and alcohol-dehydrogenase (E.C.1.1.1.1.) were also investigated, using the PAGE zymogram technique

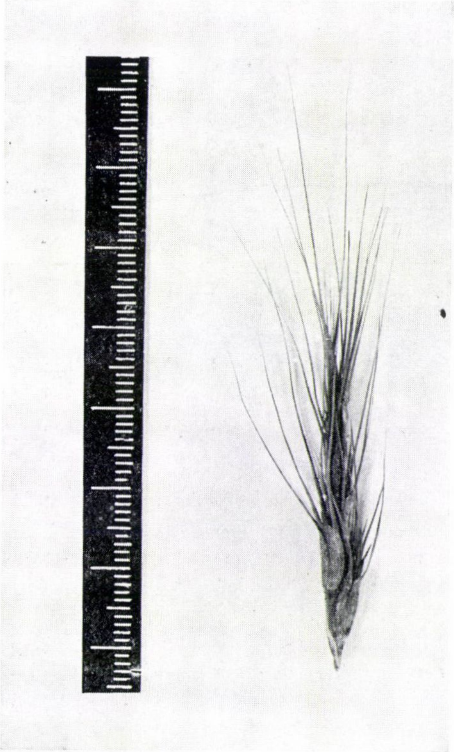

Fig. 1. F_1 ear of the *Aegilops ovata* · ssp. *carthlicum* amphiploid

Enzyme extracts were obtained from dry grains according to Honold *et al.* (1966). The grains were randomly sampled to reflect the total variation of the population, that is, the analysis did not include individual polymorphism. The samples of amphiploid consisted of the third progeny generation after treatment of the hybrid with colchicine. The PAGE method was carried out according to Davis (1964). Visualisation of esterases was achieved with the method described by Scandalios (1968), and the activity of ADH was detected using a technique adopted from Hart (1969).

Esterases. All our results concern only the so-called "fast moving", anodic (EI) esterases. As shown in Fig. 2, there are three sharply delineated bands of EI esterases in both parental species, but their phenotype differed with respect to the relative electrophoretic mobility. Bands EI-1 and EI-3 of *Ae. ovata* are electrophoretically homologous with the bands EI-1 and EI-3 of ssp. *carthlicum.* Band EI-2 in *Ae. ovata* occupies a position on the zymogram intermediate between the other two zones of activity, and has no corresponding band on the zymogram of ssp. *carthlicum.* On the other hand, ssp. *carthlicum* contains band EI-5, which is absent on the zymogram of *Ae. ovata.*

In the amphiploid five bands of esterase activity were observed. Four of these correspond electrophoretically to bands seen in the parents. The additional band EI-4, with an electrophoretic mobility intermediate between bands EI-3 and EI-5, behaves as a hybrid enzyme. This assumption is supported by the known fact that EI esterases in wheat have a dimeric structure, and that the random association of subunits into dimers results in the production of the active esterase molecule (Barber *et al.* 1968). In accordance with these facts, the observed EI zymogram of ssp. *carthlicum* can be interpreted in the following manner: the EI-1 isozyme is composed from "aa" subunits, EI-3 from "ab" subunits, and EI-5 from "bb" subunits respectively. The presence of three EI isozymes in *Ae. ovata* can be explained in the same way, assuming that one of its genomes produced "c" subunits, and the other "m" subunits ($C^uC^uM^oM^o$ genomes). Considering the fact that the "cc" esterases of *Ae. ovata* and the "aa" esterases of ssp. *carthlicum* (both EI-1) are electrophoretically identical, it can be assumed that subunits "c" and "a" are also identical. Hence the subunit composition of the five EI esterase isozymes observed is:

Esterases	Ae. ovata	ssp. carthlicum	amphiploid
EI-1	"aa"	"aa"	"aa"
EI-2	"am"	—	"am"
EI-3	"mm"	"ab"	"mm" + "ab"
EI-4	—	—	"mb"
EI-5	—	"bb"	"bb"

As it is seen, theoretically there should be six esterase isozymes in the amphiploid (Shaw 1964). Only five bands are observed, however, since the "mm" and "ab" esterases have coincident electrophoretic mobility. These results are in agreement with those described by Barber *et al.* (1968) about the EI esterases in Triticale.

Alcohol-dehydrogenase (ADH). It is known (Hart 1969, 1970, 1971) that ADH in wheat is a dimer, and that the random association of subunits into dimers results in the production of the active ADH enzymes. The gene (or genes) involved in the production of ADH is located on the chromosomes of homoeolologous group 4.

The ADH isozyme pattern observed in ssp. *carthlicum* is in full accordance with the results described by Hart (1969) in *T. dicoccum:* ADH-3 consists of $\alpha\alpha$, ADH-2 of $\alpha\beta$ and ADH-1 of $\beta\beta$ subunits.

Only one band of ADH activity was found in *Ae. ovata*. Its electrophoretic mobility is exactly the same as that of ADH-3 in ssp. *carthlicum*. Assuming a dimeric structure for ADH in *Ae. ovata*, as was proved in *Ae. squarrosa* by HART (1971), it is evident that in both its diploid genome donors, namely in *Ae. umbellulata* and *Ae. comosa*, the ADH is dimerised from identical subunits, that is from homodimers. The electrophoretic mobility of the two homodimeric enzymes is evidently identical, too. Running the mixture (1 : 1) of extracts of all three species in all possible combinations, only one band of ADH activity was observed. This provides strong support for the hypothesis of homodimeric structure. Hence it is obvious that the homodimers $\gamma\gamma$ and $\mu\mu$ of the two genome-donor species have identical electrophoretic mobility. Thus one band of ADH activity in *Ae. ovata* consists in fact of two enzymes, both of which are dimerised from identical subunits designated as $\gamma\gamma$.

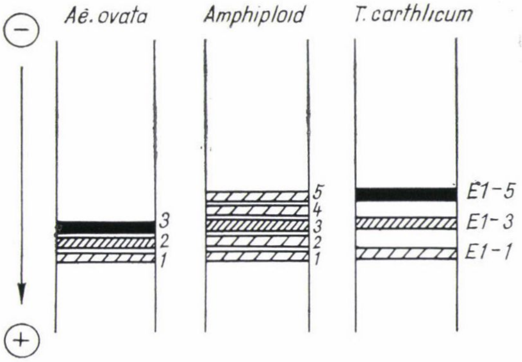

Fig. 2. Diagrammatic representation of esterase zymograms

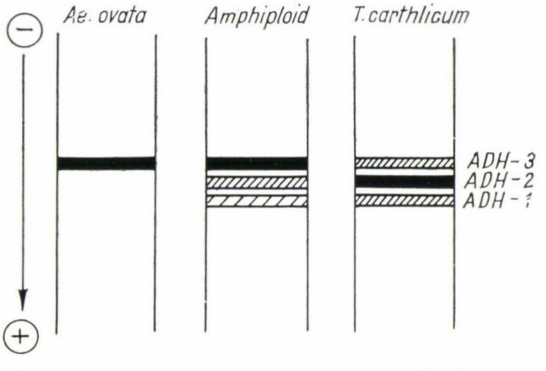

Fig. 3. Diagrammatic representation of the ADH zymograms

As is seen in Fig. 3, there are only three bands of ADH activity in the amphiploid, too. It may be noted, however, that in terms of dimeric structure, with the presence of three different kinds of subunits and of random association of subunits into dimers, there should be six ADH isozymes in the amphiploid (SHAW 1964) with the following subunit composition 1/$\alpha\alpha$, 2/$\alpha\beta$, 3/$\alpha\gamma$, 4/$\beta\beta$, 5/$\beta\gamma$ and 6/$\gamma\gamma$. The possible explanation for the three ADH isozymes is that band ADH-3 consists in fact of three isozymes with identical electrophoretic mobility ($\alpha\alpha$, $\alpha\gamma$ and $\gamma\gamma$), ADH-2 contains $\alpha\beta$ and $\beta\gamma$ isozymes, and ADH-1 is a $\beta\beta$ enzyme. A similar explanation is given by HART (1970) for the three ADH isozymes observed in *T. aestivum*.

The observation that, in the amphiploid, band ADH-3 stains much more intensely than ADH-2 and the relatively very light ADH-1 provides additional support for this hypothesis. An alternative possibility is the complete identity of subunits α and γ. In this case only $\alpha\alpha$ homodimers would be synthesised in *Ae. ovata* (as well as in the two genome donors), and the differences observed in staining intensity could be interpreted as the effect of gene dosage.

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CORRELATION BETWEEN THE RATE OF SHOOT GROWTH AND THE OPTIMUM TIME FOR BENDING DOWN THE SHOOTS IN CERTAIN FRUIT SPECIES

Within the framework of up-to-date growing techniques shoots are often bent down instead of being cut back to prevent them from becoming bare, with the additional result that the trees start to bear earlier. In many cases, however, bending down the shoots only achieves its purpose when carried out at a certain critical time (Champagnat 1965a, b). Preventing the shoots from becoming bare is also of importance both from the point of view of keeping the producing surface low and from that of slowing down the process of senescence.

The optimum time for bending the shoots down can be determined by experiments on shoots bent down at different times (Brunner 1972).

An experiment was set up to clarify whether with a simpler method, from the trend in the growth rate and the formation of secondary shoots related to it any conclusions could be drawn about differences between the fruit species as to when the tendency to become bare sets in along their shoot axes. A certain rate of growth up to a certain time is in fact able to reduce the apical dominance which affects the development of lateral buds. A frequent external sign of this is the appearance of secondary shoots on the shoot sections developing during the given period (Champagnat 1969, Brunner 1974).

Starting from this fact, we studied the course of growth in sweet cherry (Germersdorf), sour cherry (Érdi bőtermő) and apricot (Magyar kajszi) and determined which growth rates were able to inhibit the apical dominance and in which period.

In Fig. 1 the upward columns, which express the rate of growth, and the downward columns, which represent the secondary shoot formation, show that secondary shoot formation stops earliest in sweet cherry, then in sour cherry and latest in apricot (Fig. 1).

At the same time Fig. 1, and particularly Fig. 2, prove that the "utilization" of the growth rate also depends on the period of time, because in an earlier period a more intensive secondary shoot formation is induced by the same or even by a lower rate of growth than in a later period. On the other hand, the number of secondary shoots produced or ensured by a 1-mm

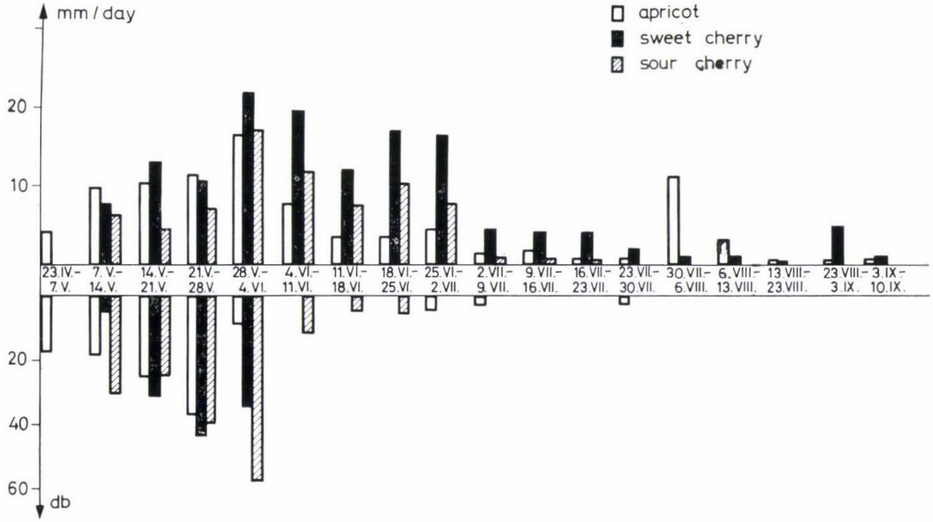

Fig. 1. Correlation between the rate of growth (upward columns) and the number of secondary shoots on shoot sections developed in the given period in 25 samples (downward columns)

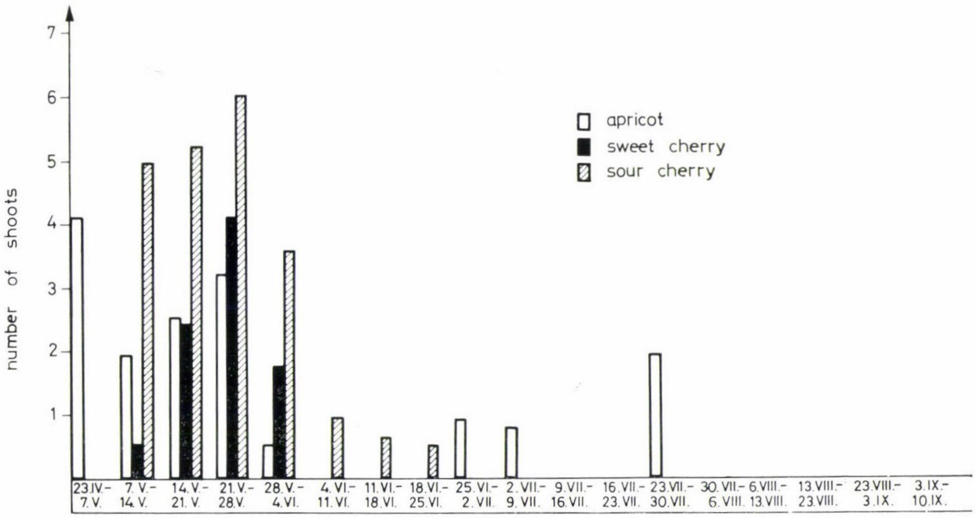

Fig. 2. Number of secondary shoots produced by a growth rate of 1 mm/day on shoot sections developed in a given period in 25 samples (Érd-Elvira, 1974)

growth rate varies with the species too. As may be seen (Fig. 2), the growth rate is utilized best by the sour cherry and for the longest period by the apricot.

From the above it can be concluded that the tendency to become bare appears first in sweet cherry, then in sour cherry and latest in apricot shoots, or more precisely, in the case of sweet cherry at the beginning of June, in sour cherry at the end of June — beginning of July, and in apricot at the end of July — beginning of August. That is, on shoot sections developing at the given times secondary shoots no longer appeared in the species (varieties) examined However, the creation of the conditions for secondary shoot formation obviously requires a greater inhibition release at the lateral bud level than does the proper development of transporting tissues in these lateral buds, which prevents the shoot axes from becoming bare the following year.

For this very reason, shoots may be bent down successfully in the same chronological order as above, i.e. first the sweet cherry, then the sour cherry and finally the apricot, but, depending on the tissue structure (maturity) of the shoots and the angle at which they will be bent, the shoots can be bent down to eliminate apical dominance some 3—5 weeks after the appearance of this tendency to become bare.

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FLOWER FROST RESISTANCE OF SOUR CHERRY, VARIETIES AND CLONES

The most important requirements of up-to-date fruit varieties are: they should show resistance to climatic factors, pathogens and pests threatening the reliability of production make possible the full mechanization of production techniques, and at the same time fulfil the quality demands raised by food industry and the consumers.

Flower frost resistance contributes to the production reliability of sour cherry varieties and thereby to an increase in their yields.

Flowers buds are most frequently damaged by frost in the spring. The earlier a fruit species (variety) blossoms, the higher the danger of frost damaging the flowers.

There are differences between the fruit species in the response of the flower to low temperatures; e.g., cherry is the most sensitive, sour cherry the most resistant of the fruit

Table 1

Extent of frost damage to flowers in parchment isolators (1974, Érd-Elvira)

Variety (clone)	Number of isolated flowers	Frost damage %	Time of full blossoming
Törpe meggy (Cigány meggy type)	767	87.6	9 April
Schattenmorelle-3 type	741	70.2	12 April
Schattenmorelle-1 type	930	67.7	16 April
Schattenmorelle-4 type	708	65.2	11 April
Latos meggy	740	48.3	16 April
Montmorency-3 type	693	40.8	8 April
Montmorency-1 type	723	34.3	7 April
Röhrings Weischel	1092	16.9	9 April
Hartai meggy (Cigány meggy type)	533	15.9	11 April
Diemitzer Amarella	1092	13.9	9 April
Montmorency Court Quevel-2 type	1382	8.4	11 April
Cigány meggy-60	451	6.2	11 April
Pándy meggy-21	923	4.9	11 April
Pándy meggy-3	3444	4.6	10 April
Cigány meggy-3	1204	3.5	9 April
Pándy meggy-11-1	2676	2.2	9 April
Cigány meggy-1317	1318	1.8	8 April
Pándy meggy-279	448	1.6	11 April
Cigány meggy-59	1325	1.4	10 April
Török meggy (Cigány meggy type)	828	1.0	7 April
Cigány meggy-215	1156	1.0	9 April
Cigány meggy-7	546	0.9	11 April
Pándy meggy-26	377	0.8	9 April

species. Frost sensitivity depends not only on the extent of chilling but also on the developmental stage of the flower.

Besides the temperature minima the extent of frost damage done to the flowers of fruit varieties is influenced by the duration of the frost, the humidity of air, the wind and its strength, the cell-sap concentration of the plant parts and the condition of the tree.

In sour cherry the threshold value of resistance is —4 °C when the buds burst, —2 °C during flowering and —1 °C for pollinated flowers. Stigmas and anthers are damaged by frost at different temperatures. The gynaeceum is damaged even between —1 and —2 °C, while the androeceum possesses a higher resistance to frost (—8 °C).

Investigations into the flower frost resistance of sour cherry varieties and clones were carried out in 1975 at the Érd-Elvira Station of the Horticultural Research Institute with a

Table 2

Extent of frost damage to uncovered flowers (1974, Érd-Elvira)

Variety (clone)	Number of flowers	Frost damage %	Time of full blossoming
Schattenmorelle-1 type	732	84.1	16 April
Latos meggy	533	42.4	16 April
Törpe meggy (Cigány meggy type)	638	34.6	9 April
Schattenmorelle-3 type	644	31.5	12 April
Schattenmorelle-4 type	577	19.0	11 April
Röhrings Weischel	606	11.7	9 April
Montmorency-1 type	555	7.7	7 April
Pándy meggy-50	931	6.0	11 April
Schattenmorelle-2 type	435	4.1	11 April
Montmorency-3 type	611	-3.9	8 April
Török meggy (Cigány meggy type)	677	3.5	7 April
Hartai meggy (Cigány meggy type)	638	2.9	11 April
Montmorency Court Quevel-2 type	899	2.6	11 April
Pándy meggy-38	769	2.6	11 April
Diemitzer Amarella	859	1.8	9 April
Pándy meggy-21	584	1.5	11 April
Cigány meggy-215	1265	1.3	9 April
Cigány meggy-60	734	0.4	11 April
Cigány meggy-1317	1361	0.2	8 April

sour cherry variety collection planted in 1955. The plantation consisted of trees spaced at 8 × 8 m, with Prunus mahaleb as root-stock, medium high trunk and crown shaped with a leader. We carried out fructification studies in the variety collection. For this purpose the flower buds were isolated with water-proof parchment bags measuring 25 × 25 cm.

In April 1974 the following radiation minima occurred at Érd-Elvira: −4.5 °C on 5th, −3.0 °C on 6th, −1 °C on 7th and −6.5 °C on 15th April. The air temperature was 0.0 °C on 3rd, and 0.5 °C on 4th and 9th April (Table 4). Temperatures measured in the parchment isolators were: −2.0 °C on 5th, −1.5 °C on 6th, 0.0° C on 7th and −2.5 °C on 15th April. In the sour cherry varieties and clones the extent of the frost damage done to the flowers was assessed on 17th April. The flower was regarded as frost-bitten when the pistil was partly or totally black. Injuries could be observed on the flowers within 12—24 hours of the frost.

In evaluating the flower frost resistance we set the following objectives:

- comparison of flowers within and without the parchment isolators for frost resistance;
- grouping of sour cherry varieties and clones according to the extent of flower frost resistance.

Extent of frost damage done to flowers with and without parchment isolators. Table 1 shows the percentage frost damage of flowers with, and Tables 2 and 3 of those without parchment isolators.

Table 3

Sour cherry varieties and clones with frost resistant flowers (1974, Érd-Elvira)

Variety (clone)	Number of flowers	Frost damage %	Time of full blossoming
Pándy meggy-279	633	0	11 April
Pándy meggy-10-1	828	0	10 April
Pándy meggy-48	687	0	7 April
Pándy meggy-114	685	0	11 April
Pándy meggy-3	1337	0	10 April
Pándy meggy-13-1	542	0	9 April
Pándy meggy-11-1	802	0	9 April
Pándy meggy-26 (Kőrösi meggy)	690	0	9 April
Cigány meggy-3	1050	0	9 April
Cigány meggy-7	517	0	11 April
Cigány meggy-59	896	0	10 April

A comparison of 23 types of sour cherry varieties and clones showed that in 20 varieties there was a higher percentage of frost damage to flowers with than to those without parchment isolators. Frost damage to the flowers in parchment isolators was 53.0 per cent higher than to uncovered flowers in Törpe meggy (Cigány meggy-type), 46.2 per cent higher in the Schattenmorelle-4 type and 38.7 per cent higher in the Schattenmorelle-3 type. There was practically no difference in the extent of frost damage between covered and uncovered flowers in the following clones: Pándy meggy-26 (0.8%), Cigány meggy-7 (0.9%), Cigány meggy-59 (1.4%), Pándy meggy-279 (1.6%), and Cigány meggy-1317 (1.6%) Of the sour cherry varieties of the Schattenmorelle-1 type examined, the flowers of Török meggy (Cigány meggy type) and Cigány meggy-215 suffered less from frost in parchment isolators (between 0.3 and 16.4 per cent) than the uncovered flowers.

Grouping of sour cherry varieties and clones according to the extent of flower frost resistance. Tables 2 and 3 are suitable for grouping sour cherry varieties and clones according to their relative frost sensitivity.

On the basis of the extent of flower frost damage we divided the sour cherry varieties and clones into four groups:

1. highly frost resistant:	0% flower frost damage,
2. frost resistant:	0.1—5% flower frost damage,
3. frost sensitive:	5.1—10% flower frost damage,
4. highly frost sensitive:	more than 10% flower frost damage.

When evaluating the frost resistance of flowers in sour cherry varieties and clones we found P-279, P-10-1, P-48, P-114, P-3, P-13-1, P-11-1 and P-26 of the Pándy meggy clones (P), and Cig. m.-3, Cig. m.-7, Cig. m.-59 of the Cigány meggy types (Cig. m.) to be highly frost resistant. The frost resistant group included: Cig. m.-1317, Cig. m.-60, Cig.m.-215, P-21, Diemitzer Amarelly, P-38, Montmorency Court Quevel-2, Hartai meggy and Török meggy (Cigány meggy types), Montmorency-3 and Schattenmorelle-2. P-50 and Montmorency-1 were frost sensitive. Schattenmorelle-1, Latos meggy, Törpe meggy (Cigány meggy type),

Table 4

Temperatures during flowering
(1974, Érd-Elvira)

Month, day	Air temperature (°C)			Radiation minimum (°C)
	maximum	minimum	daily mean	
April 1	14.5	3.0	8.7	2.2
2	14.2	1.5	7.8	6.2
2	15.5	0.0	7.8	4.9
4	17.5	0.5	8.5	4.5
5	17.5	2.0	9.8	−4.5
6	17.5	2.0	9.8	−3.0
7	17.5	5.0	11.2	−1.0
8	16.0	5.0	10.5	3.5
9	19.5	0.5	10.0	3.0
10	21.1	2.5	11.8	1.5
11	20.5	2.5	11.5	1.5
12	16.0	3.0	9.5	2.0
13	13.6	8.5	11.0	4.0
14	14.5	1.5	8.0	5.0
15	10.5	2.5	6.5	−6.5
16	20.0	4.5	12.3	4.6
17	15.0	2.5	8.7	0.0
18	13.0	6.0	9.5	2.0
19	15.5	2.0	8.8	0.5
20	16.0	0.5	8.2	4.5
21	14.5	5.0	9.7	3.5
22	13.5	1.5	7.5	−2.5
23	17.0	0.5	8.7	−2.0
April 24	14.0	1.5	7.7	1.5

Schattenmorelle-3 and -4, and the Rörhrings Weichsel varieties showed a high frost sensitivity in the flowers.

Summing up the results of the investigations we can establish that the frost resistance of flowers in sour cherry varieties and clones is a genetically determined characteristic, and its extent is very different in the varieties (clones), ranging from 0 to 84.1 per cent. Of the examined sour cherry varieties the Pándy meggy clones show an excellent flower frost resistance (being damaged in 0—6 per cent), while the frost resistance of flowers in certain Schattenmorelle types (types 1, 3 and 4), in Latos meggy and in Törpe meggy (Cigány meggy type) is very low, damage being caused in a high percentage (19.0—84.1 per cent).

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PHYSIOLOGICAL STUDIES ON SALT TOLERANCE IN PISUM SATIVUM L.
IV. TONIC COMPOSITION AND NITROGEN METABOLISM

The development of crop species and varieties suitable for growing in salt-affected soils is one of the major tasks in any programme of increasing production on such soils. This effort would obviously necessitate an understanding of the precise physiological channels through which the salt damage to crop plants was mediated, because otherwise the crop improvement would have to be based on empirical indices and hence not be so productive. Further, such an understanding would also be essential for providing the basis of special agrotechniques likely to be used on saline soils in devising methods to increase salt tolerance in plants.

In an earlier paper (Uprety—Sarin 1972b), it was reported that soil salinity affected the growth and maturation in two varieties of *Pisum sativum* L. adversely. It was also observed that the foliar spray of phosfon-D significantly counteracted the deleterious influence of soil salinization on the growth and maturation of plants. In the present communication, therefore, an effort was made to analyse the mechanism of salt effect on plants. The counteraction of salt effect by phosfon-D treatment will obviously afford a better means to provide conclusive evidence for the mechanism of salt action by reconfirming the responses through the recovery of salt stress.

Studies were conducted with *Pisum sativum* L. var. 'Rimpus'. Method of plant culture, saliuization of soil and the spraying of plants with phosfon-D was done similarly as reported in an earlier paper (Uprety—Sarin 1972b). Samples were taken from the leaves, stem and roost of the plants at 20, 35 and 50 days after sowing. They were dried at 80 °C, finely ground in a micromill and used for chemical analysis. The ground material was digested with a triacid mixture containing perchloric acid, sulphuric acid and nitric acid according to the method of Jackson (1958). The acid digest was diluted and the sodium, calcium and potassium content were determined on a flame photometer by the method described by Richard (1954). Chloride was estimated by the method of Chapman—Pratt (1960) using silver nitrate. These samples were also analysed for total nitrogen, protein nitrogen, peptide nitrogen, amide nitrogen and amino nitrogen contents by the techniques outlined in an earlier paper (Uprety—Sarin 1972a). The proteolytic activity in the leaves was determined by Yemm—Cocking's method (1965). The ribonucleic acid content was estimated according to the method of Galitz—Howell (1965) and the deoxyribonucleic acid was estimated by the method of Holden (1953).

Salt injury was manifested in the uptake and translocation of inorganic nutrients. Sodium, calcium and chloride accumulated in a large proportion in plants growing in salinized soil. Their accumulation did not vary appreciably with plant age. As compared with other plant parts, sodium accumulation was more marked in the stem and roots. The salinity-induced accumulation of calcium was comparatively more in leaves and that of chloride was more in roots. The application of phosfon-D did not bring about any marked change in the salt-induced accumulation and uptake of either sodium or calcium and chloride content. The uptake as well as the movement of potassium to plant parts was restricted by the salinization of the medium. It was also observed that the salt-induced impairment on the potassium level of plant parts was influenced neither by age nor phosfon-D treatment (Table 1).

Soil salinity considerably depressed the level of total nitrogen in the leaves, stem and roots at all the stages of growth studied. The reduction, however, was relatively more marked in the leaves followed by the stem. The application of phosfon-D ameliorated this adverse

Table 1

Effect of soil salinity and phosfon-D on ionic composition of Pisum sativum (L) var. Rimpus

Characters (Meq/100g) (dry matter)	Plant parts	Unsalinized soil			Salinized soil					
		Unsprayed plants			Unsprayed plants			Phosfon-D-sprayed plants		
		days after sowing			days after sowing			days after sowing		
		20	35	50	20	35	50	20	35	50
Sodium	leaf	17.5	18.9	20.0	35.9	37.8	36.4	34.0	37.0	39.8
	steam	20.0	22.5	23.2	51.0	54.0	52.0	50.0	55.0	58.2
	root	23.5	24.7	25.4	47.9	51.2	54.8	47.5	49.3	52.0
Calcium	leaf	329	351	287	510	559	598	523	536	550
	stem	213	273	240	252	288	300	249	300	309
	root	243	250	299	312	369	350	303	343	330
Chloride	leaf	13.4	14.1	14.5	17.9	21.3	26.0	24.3	25.9	26.3
	stem	8.3	9.1	9.5	13.9	17.5	21.0	17.1	20.0	18.9
	root	17.5	18.2	18.9	32.0	38.0	37.0	32.5	37.9	35.9
Potassium	leaf	132	140	153	92	96	108	98	100	115
	stem	89	94	115	68	75	90	75	78	90
	root	120	170	197	100	115	126	96	100	115

Table 1 (cont.)

Effect of soil salinity and phosfon-D on the absolute rate of ionic uptake in Pisum sativum L. var. Rimpus (meq./day)

		Unsalinized soil	Salinized soil	
Ions	Plant part	Unsprayed plant	Unsprayed plant	Phosfon-D-sprayed plant
Sodium	leaf	1.15	2.24	2.27
	stem	1.34	3.20	3.36
	root	1.50	3.15	3.04
Calcium	leaf	19.66	34.21	32.85
	stem	14.85	17.26	17.68
	root	16.25	21.17	20.00
Chloride	leaf	0.85	1.34	1.56
	stem	0.55	1.08	1.14
	root	1.11	2.20	2.18
Potassium	leaf	8.71	6.06	6.41
	stem	6.12	4.80	4.98
	root	10.15	7.02	6.37

effect on the leaf and stem but the changes in the roots were not marked. The salt effect was not only restricted in the nitrogen level of the plant parts but was also manifested in its further metabolism. It was observed that the level of protein and peptide nitrogen were also retarded by soil salinity. The application of phosfon-D counteracted the adverse effect of soil salinity on the protein nitrogen as well as peptide nitrogen of the leaves and stem and its ameliorative effect increased with the age of the plants. In the roots the response of phosfon-D on these nitrogen forms was not very marked. Unlike protein and peptide nitrogen there was an enhanced accumulation of amides and amino nitrogen in the leaves, stem and roots of plants growing in salinized soil. The salinity-induced accumulation of amide nitrogen was more in the roots, whereas that of amino nitrogen was more in the leaves and roots than other plant parts. The phosfon-D application lowered the amide nitrogen accumulation, particularly in the leaves and stem. Further, this treatment was also effective in reducing the accumulation of amino nitrogen in plants (Table 2).

Table 2

Effect of soil salinity and phosfon-D on various nitrogen fractions of Pisum sativum (L) var Rimpus

Characters g/100g Dry matter	Plant parts	Unsalinized soil			Salinized soil					
		Unsprayed plant			Unsprayed plant			Phosfon-D-sprayed plant		
		days after sowing			days after sowing			days after sowing		
		20	35	50	20	35	50	20	35	50
Total nitrogen	leaf	2.87	3.53	4.42	1.80	2.00	3.00	2.10	3.50	4.80
	stem	1.70	2.91	3.62	1.23	1.10	2.05	1.00	2.70	3.56
	root	2.98	3.81	3.73	2.42	2.51	2.60	2.22	2.49	2.75
Protein nitrogen	leaf	1.52	2.04	2.40	1.01	0.98	1.20	1.15	2.00	3.28
	stem	1.05	1.20	2.30	0.75	0.83	1.50	0.85	1.00	2.70
	root	1.51	1.93	1.50	1.07	1.12	1.00	1.55	1.60	1.33
Peptide nitrogen	leaf	0.237	0.280	0.365	0.159	0.163	0.257	0.208	0.378	0.565
	stem	0.135	0.150	0.182	0.096	0.125	0.110	0.100	0.119	0.138
	root	0.064	0.076	0.115	0.050	0.059	0.076	0.046	0.062	0.088
Amide nitrogen	leaf	0.102	0.125	0.138	0.159	0.177	0.203	0.124	0.143	0.157
	stem	0.081	0.120	0.135	0.134	0.197	0.231	0.141	0.190	0.200
	root	0.192	0.275	0.279	0.301	0.413	0.538	0.311	0.403	0.513
Amino nitrogen	leaf	0.172	0.205	0.309	0.198	0.272	0.395	0.182	0.200	0.250
	stem	0.123	0.170	0.192	0.174	0.220	0.270	0.180	0.190	0.200
	root	0.241	0.260	0.200	0.398	0.350	0.357	0.300	0.300	0.315

The enzymic hydrolysis of proteins in leaves as measured by proteolytic activity was higher in plants growing in salinized soil as compared to those growing in unsalinized soil. It was interesting to observe that the application of phosfon-D to plants caused a marked reduction in the salt-induced proteolytic activity in the leaves.

The salt damage was also observed in the nucleic acid levels, as the ribonucleic acid and deoxyribonucleic acid content of the leaves was considerably lowered due to soil salinity. Application of phosfon-D did not bring any improvement in the RNA level but increased the DNA level in the leaves (Table 3).

Table 3

Effect of soil salinity and phosfon-D on nucleic acids and proteolytic activity in the leaves of Pisum sativum (L) var. Rimpus

Characters	Days after sowing	Unsalinized soil	Salinized soil	
		Unsprayed plant	Unsprayed plant	Phosfon-D-sprayed plant
Ribonucleic acid	20	141	110	118
content	35	157	120	130
Mg. P/g fresh weight	50	170	135	138
Deoxy-ribonucleic acid	20	6.9	4.2	6.0
content	35	7.3	4.9	7.0
Mg. P/g fresh weight	50	8.3	5.2	8.0
Proteolytic activity	20	67	83	75
Mg. Leucine/hour/100 g	35	74	124	89
fresh weight	50	89	152	100

The salt injury on the growth and development of plants has been attributed to (a) osmotic inhibition of water uptake and (b) specific ion effect and consequent nutritional imbalances (HAYWARD—BERNSTEIN 1958). In the present study, no effort was made to analyse the osmotic and specific ion effects of salt injury. However, it was observed that the plants growing in salinized soil accumulated relatively larger quantities of sodium, calcium and chloride but failed to absorb potassium. Similar observations were made by GREENWAY (1962a) in barley and BERNSTEIN (1964) in beans. These authors observed a close relationship between the accumulation of sodium, calcium and chloride ions and the reduction in the growth and development of plants and concluded that the latter was caused by the nutritional imbalances due to the presence of these ions. In the present study with peas, no consistent relationship, with depression in growth at different stages and accumulation of either calcium, sodium or chloride ions, was observed. Further it was also interesting to observe that there was no reduction in the growth of roots (RGR), while there was an appreciable accumulation of these ions in them. In the present investigation, it was also observed that application of phosfon-D brought about a marked recovery in the growth and development of pea plants without changing the pattern of ion accumulation. These facts suggest that the results of this investigation apparently cannot lend support to the thinking that the accumulation of these ions was the primary cause of the salt damage to the growth and development of plants. The accumulation, therefore, was evidently an associated effect of salt injury to plants.

At this stage, it may be interesting to mention the results of the present finding on nitrogen metabolism. It was found that the salinization of the medium lowered the protein level in plants both by depressing the synthesis as well as accelerating the degradation of proteins. There is overwhelming evidence both by the use of inhibitors (SUTCLIFFE 1960) as also by the kinetic analysis of ion uptake (EPSTEIN *et al.* 1963) that the energy-mediated uptake of ions is achieved by proteinaceous carrier molecules and that the specific ability of plants to absorb only the required elements in appropriate quantities is achieved by the reproduction of specific carrier molecules and/or sites. Though not investigated in the present series of investigations, it has been found in this laboratory (unpublished) that the salt-induced changes in the uptake of ions are largely confined to the energy-mediated movement into the non free spaces of the roots and not the reversible movement into the free spaces. It would thus appear logical to interpret that the excessive uptake of sodium, calcium and chloride may be due to the disturbances in the ion discretionary mechanism of plants caused by the dislocation of the protein levels. It may be mentioned here that all the investigators as well as the present paper have invariably reported a lowered accumulation of potassium. The reduction in potassium has been attributed to the changes in the permeability of the cell membrane by the accumulation of calcium and sodium (HANDLEY *et al.* 1955, HOOYAMANS 1965) or by the increasing competitive effects of calcium and sodium on potassium accumulation (BERNSTEIN 1964) or the antagonistic effect of sodium on potassium levels (GREENWAY 1967b). However, in the light of the above suggestion the lowered absorption of potassium can also be ascribed to the loss of specificity of the potassium-absorbing mechanism.

It would thus appear that lowered protein level was apparently one of the primary responses of soil salinity. An analysis of this important effect revealed that similarly to germination (UPRETY—SARIN 1972a), during subsequent growth also, the block in protein synthesis was at the level of synthesis of peptide bonds, resulting in an accumulation of amides and amino acids. LAPINA (1966) in corn and STROGONOV—KABANOV (1969) in pea roots also observed a similar accumulation of amide and amono acids. However, in the presenti nvestigations it appears that the larger accumulation of amino acids may be achieved both by the lack of their incorporation in protein as well as by the hydrolysis of the proteins. The latter component is apparently achieved by the observed enhancement in proteolytic activity.

This thinking gets further support from the data on the effect of phosfon-D, wherein it was observed that this growth regulator lowered the deleterious effect of soil salinity on proteins and also reduced the salt-induced accumulation of amino nitrogen. The ameliorative effect of phosfon-D on the growth and development of pea plants, therefore, appears to be mediated by its capacity to improve the protein level by reducing their hydrolysis as also by increseing the peptide synthesis.

The present finding suggests that the reduction in the level of proteins is one of the primary damages experienced by plants growing in salinized soils. This protein depletion manifests itself in altering both the organic and inorganic make-up, thus resulting in a reduced growth and yield of the plants. The ameliorating agent phosfon-D, which helped in maintaining the protein status of plants, is able to counteract the adverse effect of soil salinity on the growth, development and yield of plants.

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"NAGYSZÉNÁSI" LUCERNE

Taxonomical place: *Medicago sativa* L.

Origin: selected from stands found in the neighbourhood of Nagyszénás (southern part of the Great Hungarian Plain).

Beginning of breeding: 1951, Martonvásár.

Breeders: Dr. Andor Jánossy, Zoltán Csák, Dr. Zoltán Böjtös; variety maintainer; Dr. Gábor Kovács, Szarvas.

State qualification: first accepted in 1952, state certified variety 1961 (Kapás *et al.* 1965).

General characterization: a vigorously sprounting variety not inclined to lodging, maintaining a thick stand even for 4—6 years, with abundant foliage, fine shoots, excellent feeding value, drought and frost resistance, and high productivity.

Morphological description:

Root system: the strong rhizome develops abundant roots.

Shoot system: 60—70 cm high; the number of plants in the first year is 47—51/m thinning to 16—18 by the fourth year (Kiss 1968).

Stem: while thin and of fine structure, still firm, slightly undulate.

Foliage: the percentage of leaves on the shoots is 49.4; leaves are thickly set not only on the laterals but also on the main shoot; the leaflets are large, elongated elliptical, of medium green colour, finely indented at the leaf apex.

Flowers: the corolla is generally dark blue, but can be violet and sometimes even light blue coloured. Flowers are thickly set in the clusters.

Pod: number of seeds per pod: 2.7—4.9

Seed: thousand-grain-weight: 1.8—2.5 g.

Biological characters:

Vegetation period: it forms a closed stand for 4—6 years. When sown early (at the end of March) it comes up after 13—14 days, but sowing at the beginning of May — which corresponds to the ecological requirements of the variety — results in emergence in four days. In the year of sowing flowering occurs at the end of June —beginning of July (when sown in May, around 10 July). The following year, after a favourable overwintering period, it sprouts excellently

Water requirement: tolerant to drought.

Resistance to disease: fairly good.

Farm technology requirements:

Seeding: its genetic character is best suited by sowing in the first decade of May when the temperature at the depth of sowing is 18.8 °C (Mándy 1972).

Soil requirement: with the exception of sandy soils it can be grown in any soil with a satisfactory nutrient level (Kapás *et al.* 1965).

Productivity: the amount of green yield ranges from 220 to 525 q/ha (Csák—Kiss—Szerafin 1964). In the variety trials the total green yield of four years was 1667.9 q/ha. The crude protein content of the green yield is 18—22 per cent, the protein output 15—23 q/ha, except for the year of sowing when it is only 2 q/ha (Kiss 1968).

Area of cultivation: it can be grown everywhere in Hungary in fairly good soils.

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LECTIONES

DATA ON HETEROSIS BREEDING AND PREDICTION OF HETEROSIS EFFECT IN CATTLE BREEDING*

The effect of heterosis or hybrid vigour is utilized throughout the breeding of animals especially in poultry and pig breeding. It has been used for a long time also in cattle breeding, although there are many unknown details both in the practice and in research, and development in this field has been slower than expected. The cause may be the low reproduction rate, the high financial value of the individuals, the sensibility for inbreeding and other not yet precisely determined conditions which are all limiting factors in exploiting the advantages of heterosis breeding.

According to MASON (1966) heterosis "is measured as the superiority of the mean of reciprocal F_1 crosses over the mean of the two parental breeds". The former definition of hybrid vigour, supposing that the parents are homozygous, is not adaptable for quantitative traits.

Heterosis can be exploited by the system of various types of crossings (commercial crossing between two breeds, crisscrossing, topcrossing) and even by the combination of two lines within one breed (MASON 1966, HORN—DOHY 1969).

From a practical point of view it is important to know, that hybrid vigour can be expected only in such characteristics which have low heritability estimates like reproduction, vitality, growth rate, milk, butterfat and protein quantity, respectively.

Concerning the problems of the heterosis breeding of cattle let us take first the results of milk production. It may occur, that the heterosis effect appears where it has not been expected as shown in Table 1.

Table 1

Demonstration of an unexpected heterosis effect in butterfat quantity

Breed	Milk, kg	Butterfat, %	Butterfat, kg
A	6000	3.5	180
B	3000	6.0	180
F_1 (A×B)	4500	4.5	*202.5*

(HORN—DOHY 1970)

Referring to Table 1, no heterosis effect appears in milk quantity and butterfat per cent, yet there can be found a superiority in butterfat quantity (HORN—DOHY 1970).

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* This paper was presented on the 7th Conference of Animal Genetics held at Tatranska Lomnica (Czechoslovakia) in June 11—14, 1974.

As the absolute milk production shows the quantitative level of production, the relative milk production (related to live weight) is the indicator of effectiveness. From this point of view it is worth considering the rate of hybrid vigour in relative milk production. It has been stated that the advantage in this field related to the absolute production in the F_1 population can be doubled.

In general, we speak of a "positive" heterosis effect, but it can also be negative as demonstrated in Table 2.

Table 2

Demonstration of negative heterosis effect

	I. parent population	II. parent population	F_1 population
Milk production, kg	4000	5000	4500
Live weight, kg	400	800	600
Milk prod./100 kg live weight	1000	625	*750*
Expected relative milk production on the basis of parents' relative milk production (1000 + 625) : 2 =			*812*
Result of negative heterosis effect in rel. milk prod.			— 62 kg

(Dohy 1973)

In connection with beef production in suckler cow herds there are some special problems to be solved such as improvement of reproductive traits in the dams and body conformation, slaughter value in the calves fattened. There is a contradiction between the selection criteria for suckler cow and feedlot cattle, which was first emphasized by Cartwright (1971) and can be solved only by a combination between different breeds (Drögemeier 1971a, b, c, Kelemér 1973).

The most common methods provocating hybrid vigour in beef cattle are as follows: crossings between dual purpose breeds, crossings between beef breeds, crossings between dual purpose and beef breeds and the crossings between dairy and beef breeds. The highest heterosis effect can be expected by the method of crossbreeding between dairy cows and beef bulls, while the lowest one between dual purpose and beef breeds or within dual-purpose breeds (Mason 1966). Mason pointed to the high effectivity of crossing between different beef breeds which was realized in a higher number of weaned calves (18—25% more calves) and this has been confirmed also by Smirnov *et al.* (1973).

A high effect can be expected from the method of topcrossing which is shown on a model formed by Horn—Dohy (1969) — (Fig. 1).

The main point of this method is the combination of a controlled inbred male line with a non-inbred female line provocating in this way a high hybrid vigour. Practically it can be reached by keeping a good suckler cow herd resulted from a crossing between dairy cows and beef bulls and then mating them to the male line in which the required positive genes are accumulated by inbreeding. This method belongs to the three-breed-crossing and seems to be the most practical and most effective one at the same time. The utilization, in this context, of heterosis and additional economic gain lies in the fact that an economic calf producer (the female line) is combined with an efficient male line.

Through these data the importance of the heterosis effect and some aspects of its realization in the breeding practice have been demonstrated. We may draw the conclusion that hybrid vigour is due to heterozygosity in the crosses in association with good combinations of positive genes. This means that we may expect a higher effect of heterosis if a higher heterozygosity can be achieved in the offspring (Cress 1966). This is the basis of predicting the heterosis effect according to our conception.

For the prediction of the expectable heterosis effects immunogenetics, though indirectly, also offers some suitable information.

An immense amount of data are accumulating in blood grouping laboratories on the genetic structure of various cattle breeds all over the world. If these informations, regarding nearly 20 different loci, are systematically analyzed by means of especially developed statistical methods, we can achieve a valuable basical knowledge about the cattle breeds we are working with.

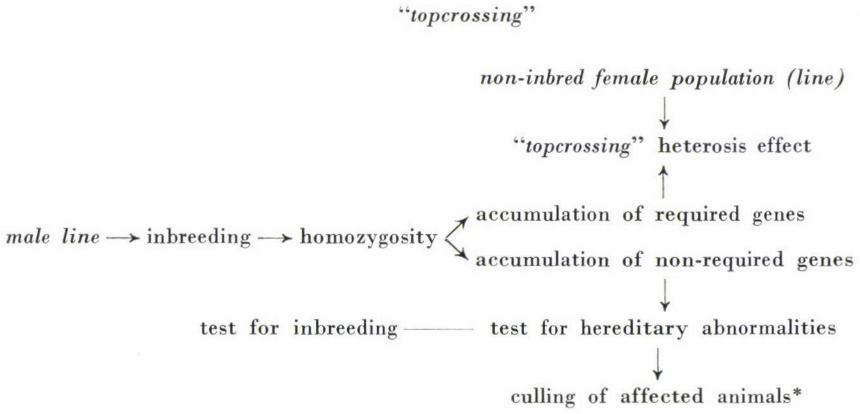

Fig. 1. "Topcrossing" (* It has a special importance when F_1 individuals are recrossed with the male line), (Horn—Dohy 1969)

When thinking at a population level the ratio of individuals homo- or heterozygous for a limited number of bloodgroup loci, can be calculated from the relative gene frequencies. In this way from a choice of breeds we can select those with the highest ratio of homozygosity — possessing no or only a few genes in common and by crossing them the highest heterosis effect can be reached.

Thus, by taking immunogenetic traits into consideration in the case of two breeds or populations to be crossed, the expectable heterosis effect can be estimated on the basis of the relative gene frequencies. It is important that the same loci be considered in the breeds concerned. In cattle the most polymorphic B blood group locus offers itself as the most suitable one for such estimations (see Table 3) but in general the more loci the better.

If we want to find a crossing partner for the Danish Friesian (SDM) breed from among the breeds included in Table 3, theoretically any of the breeds can be chosen except the Dutch Friesian (FH) one. The latter has 4 alleles in common with the Danish Friesian (SDM) breed, therefore a certain relationship can be supposed between these two breeds. Of course, in the selection of the actual crossing partners several other production characteristics must also be considered. A heterosis effect can also be expected if the ratio of homozygous individuals within one of the breeds to be crossed is relatively low, but they have no or only a few alleles in common (Table 4).

Table 3

5 most frequently occurring B-phenogroups in 9 different cattle breeds

Ayrshire			Danish Friesian (SDM)			Dutch Friesian (FH)		
O_1A'	0.22		I'	0.19		$BGKO_3Y_2A'$	0.12	
BO_1Y_2D'	0.14		$GY_2E'_1$	0.12		$GY_2E'_1$	0.10	
O_1	0.11		$BOY_2A'E'_3$	0.11		I'	0.09	
$O_xY_1E'_3G'Y'$	0.10		E'	0.04		E'	0.08	
$O_3QJ'K'O'$	0.04	0.61	PI'	0.03	0.49	$BOY_1A'E'$	0.07	0.46
Holstein Friesian			**Danish Jersey**			**Hung. Fleckvieh**		
$GY_2E'_1$	0.21		$BGKOY_1A'E'K'$	0.17		G_1A'	0.11	
$O_3J'K'O'$	0.06		BG'	0.16		O'	0.07	
$O_xD'E'_3F'G'O'$	0.06		Y_1D'	0.15		$Y_2A'D'E'_2$	0.06	
$O_xE'_3$	0.06		$QD'E'_1$	0.11		$G_3O_1T_1E'_3K'$	0.05	
O_1A'	0.05	0.44	E'_1	0.09	0.68	O_2	0.04	0.33
Swiss brown			**Danish red (RDM)**			**Hungarian grey**		
$O_1T_1Y_2E'_3F'$	0.37		Y_2	0.21		$BG_2KE'_2O'$	0.23	
$BO_2Y_2A'E'_3G'Y'$	0.10		BO_1Y_1D'	0.20		$G_3O_1T_1E'_3K'$	0.16	
$BPY_2G'Y'$	0.05		BO_1	0.18		$G_3PQA'D'$	0.09	
O_xO'	0.05		B	0.13		$Y_2A'B'D'E'_3G'$	0.09	
$BGKO_xE'_2F'O'$	0.04	0.61	$QOJ'K'$	0.05	0.77	b	0.08	0.65

(Kovács 1968)

Table 4

Heterosis effect in Jersey ♂ × Hungarian Fleckvieh ♀ F_1 population concerning the average milk production of 1972—73

Breeds	n	Live weight, kg (estimated)	Milk kg	Butterfat %	Butterfat kg	FCM kg	FCM/100 kg live weight
Jersey	16	450	3229	6.22	200.7	4295	954
Hung. Fleckvieh	180857	650	2950	3.82	112.6	2862	427
Average of parents' production			3090 (100%)	5.02 (100%)	156.6 (100%)	3579 (100%)	691 (100%)
F_1 population	6148	520	3376 (109%)	4.75 (95%)	160.2 (102%)	3781 (106%)	727 (105%)

(Keleméri 1973)

In the near future the heterosis effect can be better exploited in cattle breeding when the investigations bring more details up to the surface in sex ratio determination, egg transplantation and the physiological background of the heterosis effect.

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GROWTH REGULATING EFFECT OF CASEIN HYDROLYSATES AND RIBOFLAVIN ON TOBACCO CALLUS TISSUES*

It is a well-known fact that some culture media components act as "secondary" regulators and also affect the interactions of the "primary" regulators (stimulators and inhibitors) in the growth of plant tissues. Certain casein hydrolysates and vitamins can also act as such "secondary" regulators (Hildebrandt 1962, Murashige—Skoog 1962). In our experiments we examined the regulating effects of amparon, aminosol, aminofusin (casein hydrolysates) and riboflavin (vitamin) as "secondary regulators" (Maróti 1974).

Amparon *(Am):* 5% protein hydrolysate + 10% saccharose (Organon, Oss. prep.)

Aminosol *(As):* 3.3% dialysed casein hydrolysate + 5% glucose (Vitrum prep. Stockholm).

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* Demonstrated at the 3rd International Congress of Plant Tissue and Cell Culture, Leicester (England), 1974.

Aminofusin *(Af)*: 50 g/l amino acid + 100 g/l sorbitol + vitamins (ascorbic acid, niacin, riboflavin, pyridoxine, meso-inositol, rutin-$NaSO_4$). (J. Pfrimmer and Co. Erlangen; Pharmazeutische Werke prep.).

Riboflavin *(RF)*, thiouracil, (*TU* inhibitor).

«Primary» regulators: indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin (K), gibberellic acid (GA).

Test material: Callus tissue cultures of tobacco *(Nicotiana tabacum* L.)

Basic medium: MURASHIGE—SKOOG (1962) macro- and microelements (M-S) with vitamins and amino acids (aneurin 1 mg/l, niacin 0.5 mg/l, pyridoxine 0.5 mg/l, meso-inositol 100 mg/l, glycine 2 mg/l);

GANTHERET (1959) macro- and Hoagland "A-Z" microelements (G+H) with amino acids (cysteine 10 mg/l, glycine 2 mg/l);

The ferric component was given in the form of Na_2Fe-EDTA (3 ml/l) to the nutrient medium solidified with agar (0.8%).

Before autoclaving, the pH was set at 5.7. The carbon source was 3% saccharose (MARÓTI 1970, 1971, 1974).

Incubation: 4 weeks, in darkness, at 28° (±1) or 20° (±1) C.

Methods: The experiments was carried out on the basis of our earlier work (MARÓTI 1970, 1971, 1974, SZIRÁKI—MARÓTI 1973).

Cell number, determination according to BROWN—RICKLESS (1950).

Protein, determination according to PILET—BRAUN (1967). RNA, determination according to LOWRY *et al.* (1951). The results presented here are the arithmetical means of five parallel measurements, different from each other in no more than 10 per cent.

The ratio of the hormonal constituents of the basic medium resulted in a weak tissue growth only, but the cell number, calculated to a unit weight, was relatively large (Fig. 1).

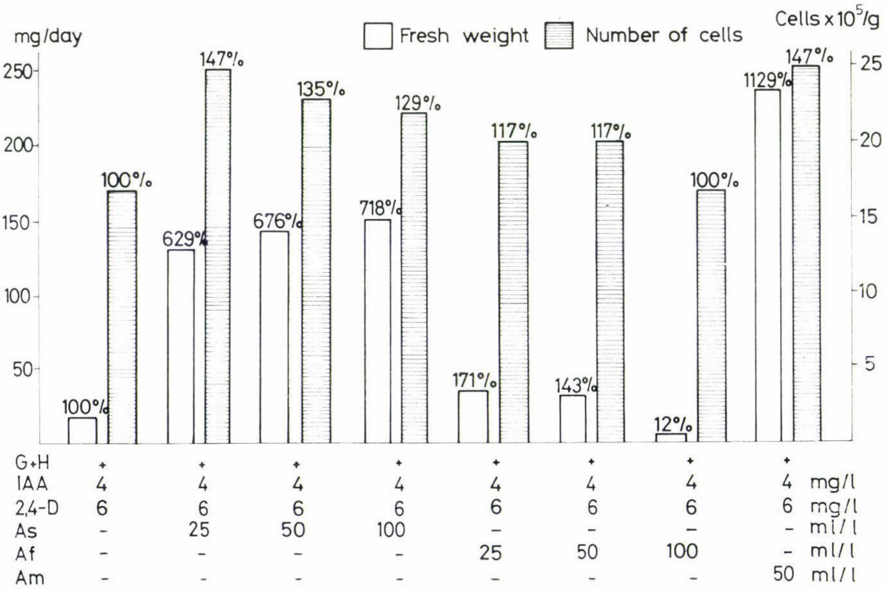


Fig. 1. The effect of IAA, 2,4-D and casein hydrolysates on weight increase and cell number of tobacco tissue cultures (Incubation: 4 week, in darkness, at 20° (±) 1C)

The *As* increased the intensity of the tissue growth as a function of the increase in concentration. The cell number also increased in inverse proportion to the increase in the concentration. The *Af* only stimulated the growth of the control tissue in the two smaller concentrations. The largest concentration used actually inhibited the growth of the tissue. The cell number here was also bigger than in the control. The *Am* resulted in a peculiarly big growth stimulation and the cell number was big too.

This ratio of hormone combination (Fig. 2) resulted in a big tissue growth. The *As* used diminished this intensity of growth. The inhibition of the growth increased with the concentration. On the other hand, the *As* slightly increased the cell number. The effect of the *Af* on the growth of the tissue weight was smaller than that of the basic medium. With the increase of the concentration of *Af*, the tissue weight decreased. The cell number was nearly equal to the control. The effect of *Am* best approached the results of the control.

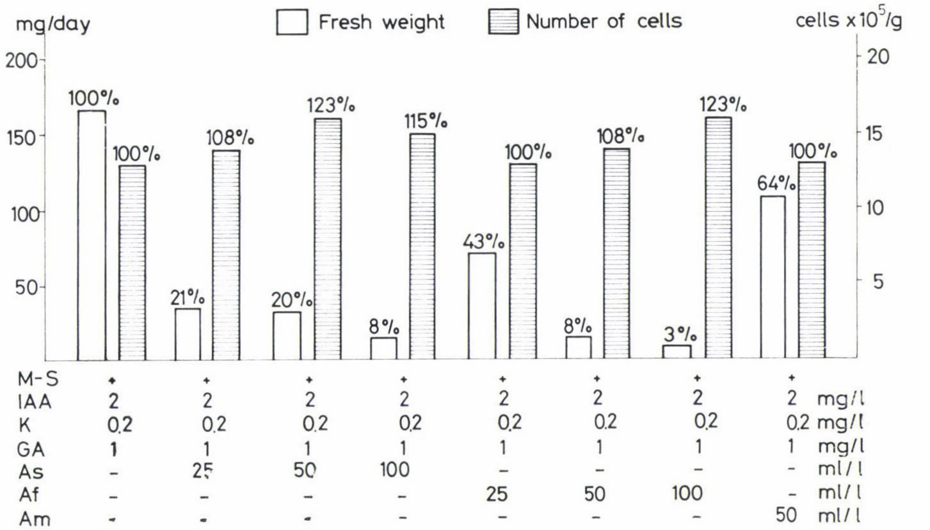


Fig. 2. The effect of IAA, K, GA and casein hydrolysates on weight increase and cell number of tobacco tissue cultures. (Incubation: 4 weeks, in darkness, at 20° (±1) C)

The hormone combination of the control medium resulted only in a weak growth of tissue (Fig. 3). The *RF* stimulated the growth of the weight, but this was not proportional to the rise in the concentration. Also, the protein and RNA content of the cells increased with the rise in *RF* concentration. *RF* seemed to have a direct effect on the quantitative change of RNA.

The smallest concentration of *TU* used did not result in any change, either in the growth of weight or in the protein and RNA content of the tissues (Fig. 4). In the bigger concentrations, however, this inhibitor inhibited both the increase in weight and the synthesis of protein and RNA.

The most intensive stimulating effect of the *RF* was antagonised by *TU* in accordance with the growing concentrations used (Fig. 5). The *RF* was able to compensate for the inhibition of the *TU* both in the protein synthesis and in the synthesis of RNA, but not at an equal rate.

It is known, that the ratios of the hormonal compounds regulate the growth of plant tissues. Therefore, the interaction of certain stimulators can result in inhibition too. The effect

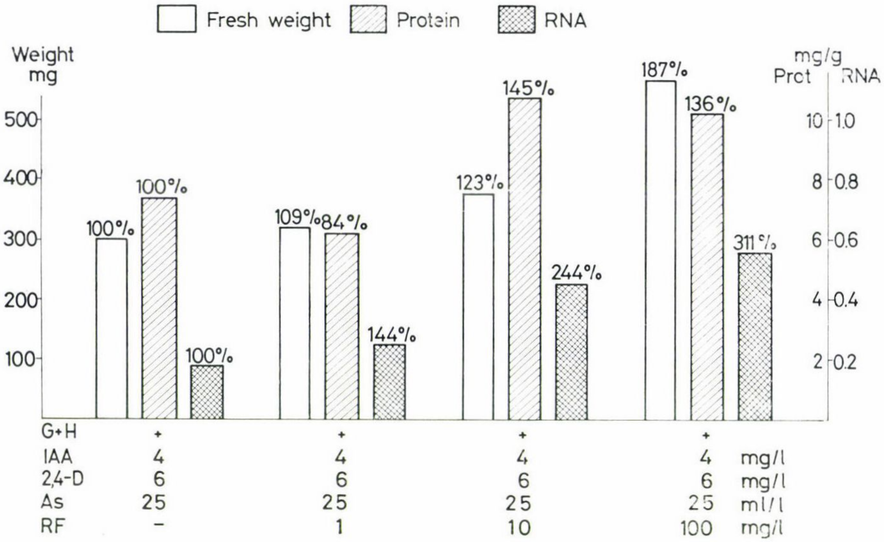


Fig. 3. The effect of IAA, 2,4-D and Riboflavin on weight increase and Protein, RNA content of tobacco tissue cultures. (Incubation: 4 weeks, in darkness, at 28° (±1) C)

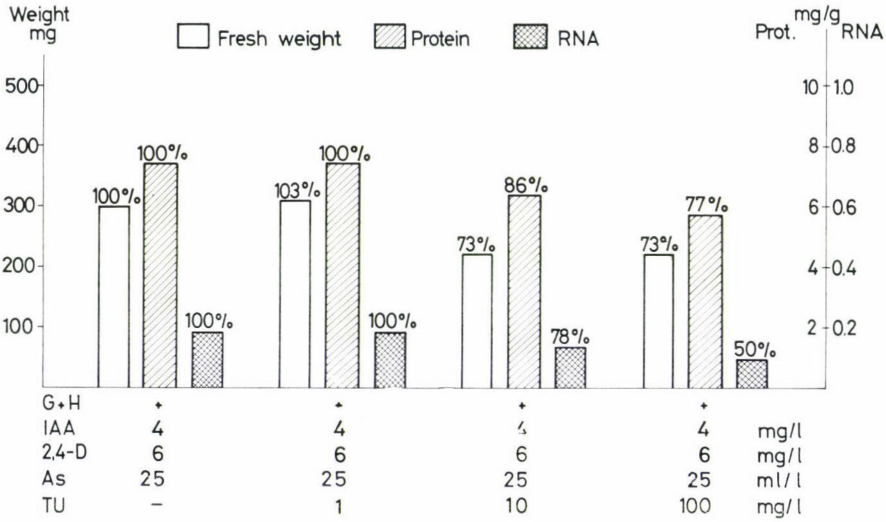
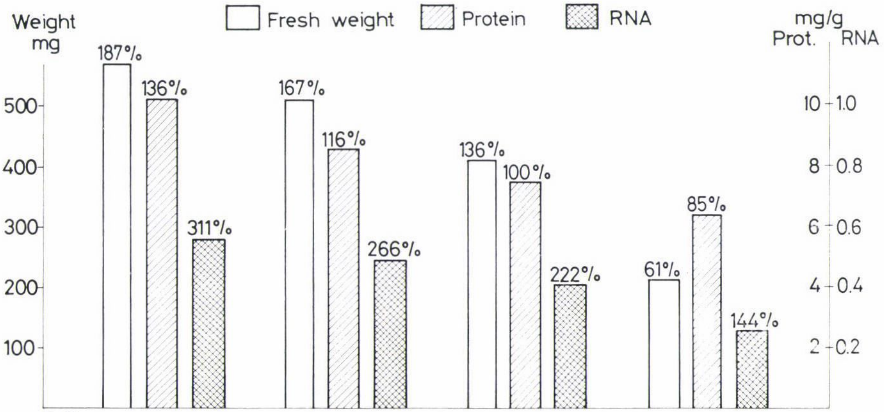


Fig. 4. The effect of IAA, 2,4-D and Thiouracil on weight increase and Protein, RNA content of tobacco tissue cultures. (Incubation: 4 weeks, in darkness, at 28° (±1) C)

of IAA and 2,4-D used simultaneously proved this in Gantheret's basic medium. Some sorts of casein hydrolysates like *Am*, *As* and *Af* antagonised the interactions of these "primary" regulators. Stopping the growth inhibition is not caused by these at an equal rate however. The inhibition in the growth of the tissue was antagonised best by the *Am*, which contains protein hydrolysate and saccharose, and least by the *Af*, which contains amino acids and sorbitol. The number of cells in the unit weight increased in every variant according to the effect of the casein preparations. From this we can deduce relatively less weight and an intensive division of the cells.

The combination of hormones (IAA, Kinetin, GA) in Murashige and Skoog's medium produced a more intensive tissue growth, which also indicated a better ratio of hormones necessary for the growth. In such a hormone ratio the casein hydrolysates acted not as a stimulator, but as an inhibitor. The number of cells increased in these variants in the unit weight. Hence the casein hydrolysates were able to compensate for the unfavourable hormone ratio to the increase or for the antagonism of the regulators and they could inhibit the effect of growing in a favourable hormone ratio. These results suggest that the casein hydrolysates — in accordance with the literature (Hildebrandt 1962, Murashige—Skoog 1962) — should be regarded not only as an N source and nutrition material, but as supplementary or "secondary" regulators in tissue growth.



G+H	+	+	+	+	
IAA	4	4	4	4	mg/l
2,4-D	6	6	6	6	mg/l
As	25	25	25	25	ml/l
RF	100	100	100	100	mg/l
TU	-	1	10	100	mg/l

Fig. 5. The effect of IAA, 2,4-D and Riboflavin, Thiouracil on weight increase and Protein, RNA content of tobacco tissue cultures. (Incubation: 4 weeks, in darkness, at 28° (±1) C)

The *RF*, which belongs to the vitamins, also stimulated the growth of the tissues with the interactions of some regulators. On the Gantheret's medium completed with IAA, 2,4-D and *As*, the *RF* produced an increase in growth as a function of the rise in concentration. The change of the protein and RNA content indicated the tissue growth too. However, the *TU*, known as a uracil antagonist, inhibited the growth of the tissues in the same medium and this antagonised the largest-scale stimulation of *RF* as well. Both the inhibition of the *TU* and the antagonising of the *RF* stimulation were virtually reflected in the decrease of the protein and RNA content too (Khan 1967, Schrandolf—Legler 1969).

It can be concluded, that the change of the tissue weight was an immediate consequence of the change in the protein and RNA content. From the known protein and RNA synthesis inhibition and from the interaction of the *TU* with the *RF* it is supposed that the stimulating effect of the *RF* is directly connected with the change of these two compounds too. The above-mentioned fact supports the supposition that *RF* can act as a regulator in the tissue growth (Sharma—Datta 1959, Wollgiehn 1965, Wollgiehn—Parthier 1964).

Our results suggest that the concept and territory of compounds regulating tissue growth should be extended to such "secondary" regulators as casein hydrolysates and vitamins.

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FORUM

DIFFERENCES AND CORRELATIONS BETWEEN THE BIOLOGICAL VALUE AND IN VITRO EXAMINATION RESULTS OF PROTEINS

Human and animal life is based on the balance of the building up and breakdown of proteins composing the organism; this is ensured by a regular protein uptake. A part of the amino acids released from molecules broken down by enzymes from proteins incorporated in the organism will be synthetized into molecules of new proteins, while another part of them further decomposes into urea, carbon dioxide and water with a simultaneous energy release (Karlson 1972). The new protein molecules incorporated in the organism are specific to species and the amino acids building up the same protein and the order in which they are used are genetically determined; thus the amino acid composition of proteins of the same character and function is not quite identical in the different species. Therefore, the biosynthesis of the same proteins requires more or less different quantities and qualities of amino acids.

A part of the amino acids required for the biosynthesis of proteins are produced by the organism itself, but some of the necessary amino acids are obtained from proteins introduced from outside; the demand for these so-called essential amino acids is to some extent also specific. The essential amino acid requirement of one animal species, e.g. rat, will not thus agree with that of man or of another animal species with a simple digestive system (Jekat—Pabst 1969). (This difference is even more apparent in the microbiological "utilization" experiments.)

Protein biosynthesis in the organism thus raises certain demands on the quality of the introduced protein. Therefore, when studying and evaluating the proteins of human food and animal feed, we must take into consideration that utilisation does not only depend on the extent to which the proteins can be decomposed and the quantity of other food or feed components (carbohydrate, fat, salts, microelements, vitamins, indigestible fibrous components) provided together, but also on the species and abilities of the individual examined and on conditions influencing protein synthesis. It is obvious that the utilization of a protein in humans or in an animal species can only be pointed out by in vivo utilization experiments carried out with humans or the respective animal species under exact conditions. It should be emphasized that the term "exact conditions" includes not only the methodological precision of the protein analysis and the correct accomplishment of sample taking both of food (or feed) and excretions, but also, in the utilization experiments, the natural conditions corresponding to the conventional way of life provided for the animal. Results of experiments using a fistula prepared by operation, or animals kept in a so-called utilization pen are of questionable value, because the stress suffered by the animal, as well as psychosomatic changes and mental processes manifested through the vegetative nervous system, greatly affect the functioning of the digestive system.

The requirements outlined above were fully met by Kofrányi and his team in long-term nitrogen balance experiments carried out on humans for more than ten years at the Max-Planck-Institut für Ernährungsphysiologie, Dortmund, with a so far unprecedented scientific care and presicion. A basic importance of the experimental results, published in 18 parts, is their nullifying generally accepted theses like the principle of limiting amino acids, further the sophistic values of "essential amino acid indices" and "chemical score" (KOFRÁNYI—JEKAT 1964a). Kofrányi determines the biological value of proteins on the basis of the amount required for maintaining the nitrogen balance of the organism, calculating the values by g protein · body weight $kg^{-1} \cdot day^{-1}$, that is, the higher the biological value of a protein the lower the amount of protein required for maintaining the nitrogen balance.* In the experiments performed by him the amounts fulfilling the minimum requirement and the amino acid composition of proteins were not in agreement with the ideas about essential amino acid index and chemical score, since foodstuffs given in pairs when mixed at certain ratios proved to be of higher biological activity than pure eggwhite, which had previously been considered as the optimum. Accordingly, until a reliable experimental disproof of Kofrányi's experimental results is published, we consider the discussion of various limiting amino acid theories and theoretically obtained indices dispensable.

Notwithstanding that the biological value of proteins can only be determined by in vivo experiments carried out with the above described principles and technical solutions, the difficulties and high error limits of both human and animal in vivo experiments, as well as the wish to acquire a more thorough knowledge of the food and feed proteins (partly for economic and commercial reason), motivated the efforts to findin vitro chemical and biochemical evaluation methods suitable for their qualification too. A part of the laboratory in vitro methods used for the qualification of food and feed proteins are simple protein analyses whose results give direct information about one or other property of the examined material. The biochemical in vitro techniques used to study the protein decomposition are complex methods based on the physiological phenomenon that in the human organism, and in that of animals with a simple gastric system, the decomposition of the protein molecule is the first step in protein conversion.** In in vitro examinations the protein is likewise first decomposed by enzymatic splitting, then the products of proteolytic splitting are submitted to various analyses.

What we regard as a method providing results suitable for immediate evaluation are the nitrogen determination and amino acid determination.

By multiplying the result of nitrogen determination with an appropriate conversion factor we obtain the approximate protein content (incorrectly: "crude protein" content) of the material examined, which may serve for its basic characterization.

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* About the methodology of the experiment, described in a separate publication (KOFRÁNYI—JEKAT 1964b) with full particulars, we only mention the following: The persons (mostly university students) participating in the experiments continued to live in their usual way in a kind of students' home. In the experiments nitrogen content was determined not only in the consumed food but also in all substances introduced into the organism, e.g. tea, chewing gum. And when calculating the amount of nitrogen discharged, nitrogen loss involved in the growth of nails and hair, the detachment of the epidermis and in perspiration was also taken into account. In the column "Notes" of the experimental persons' diary unusual events like 5 km walking, 90 minutes playing handball, or headache, tiredness, etc. were registered. The experimental persons were kept under constant physiological and medical control, including the determination of alanine-transaminase and aspartate-transaminase content in the serum: changes in the ratio of the latter two is indicative of lesions in the parenchyma cells of the liver, originating from a deficient amino acid supply. The extraordinary preciseness of the methodology is shown by the limit of error of parallel experiments being within ± 1.5% (KOFRÁNYI—JEKAT 1964c). More than 200 long-term (minimum half a year each) nitrogen balance experiments were carried out with 38 persons for more than ten years.

** Since animals with a compound gastric system obtain their protein requirements from bacterial proteins through the anaerobic microbial decomposition and conversion of protein, cellulose, starch and non-protein nitrogen (urea, biuret), only the physiological processes of man and of animals with simple gastric systems can serve as a model for studying the value of proteins.

From the amino acid determination of the analytic material we obtain the amino acid composition of component proteins and peptides and the amount of free amino acids possibly present. This determination is important, if only because the basis of the biological value of food and feed proteins is the relative quantity of the individual amino acids. Although the importance of the ratio between the component amino acids and its effect on protein conversion are mostly unknown, from the data of total amino acid determinations we still get a picture of the protein composition in the analytic material, as well as of the actual quantity of protein.

In vitro protein decomposition should be, in fact, a copy of natural conditions in order to obtain the truest possible picture of the first phase of protein metabolism. In the methods found in the literature the ways of choosing and using the enzymes and of studying the products of protein splitting are more or less different (Table 1).

Table 1

Enzymatic in vitro methods

Enzymes used	Author
pepsin	SHEFFNER—ECKFELDT—SPECTOR (1956)
pancreatin	MENDEN—CREMER (1966)
pepsin, pancreatin	AKESON—STATMANN (1964)
pepsin, pancreatin	PRAHL—TÄUFEL (1967)
pepsin, pancreatin, trypsin, erepsin	FORD—SALTER (1966)
pepsin, papain	FORD—SALTER (1966)
pronase	FORD—SALTER (1966)

On the basis of many years of experience, taking the works of FORD—SALTER (1966) into consideration, we have developed an examination method by means of which — according to our opinion — results providing sufficient data on the decomposition of the material of analysis can be obtained.

I. *Chemical routine analyses serving the general characterization of food and feed proteins**

1. Nitrogen determination. The method to be used depends on the quality of the sample, the possibilities of deaggregation and the instrumental level of the laboratory, with the stipulation that the result of the methodology chosen should agree with the actual nitrogen content. As for choosing the analytically best method (with the specifications of standards serving commercial purposes left out of consideration), detailed information can be obtained from Bradstreet's monograph (BRADSTREET 1965).

2. Amino acid determination with automatic analyser (SPACKMANN *et al.* 1958). The 6 N HCl hydrolysis is performed as specified by MOORE—STEIN (1963), depending on the quantities of substances (primarily carbohydrate) other than protein at a ratio of 1 : 1,000—1 : 5,000.

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* Since the description and discussion of the methodology of analyses is neither the task nor objective of this publication, but on the other hand, the knowledge of the details and technical data of the methodology used is important with a view to their successful application, a detailed description of them is given in a separate publication.

For the precise determination of the amounts of Cys and Met, performic acid oxidation is carried out on a separate sample of the analytic material according to Hirs, then the cysteic acid and methionsulphoxide obtained are chromatographed (HIRS 1956).

With the purpose of determining Trp, $Ba(OH)_2$ hydrolysis is carried out (NOLTMANN *et al.* 1962), then chromatography is applied according to Moore and Stein.

II. *Study of food and feed proteins in vitro on the basis of their enzymatic decomposition*

A) *Description of enzymatic decomposition*

1. Decomposition with pepsin. A sample containing maximum 1 g protein is kept at 38 °C for 15 minutes, then 25 mg 1 : 10,000 pepsin is added to it. The lightly stoppered glass is kept for 4 hours in an ultrathermostat at 38 °C while constantly shaken. After this the undissolved part is centrifuged for a few minutes at 2,000 g, then the solution is decanted from the residue and diluted to 50 ml with distilled water. Of the solution, 2.5 ml is measured out for analysis; this sample is kept in cold storage until it is used.

2. Decomposition with trypsin. After decomposition with pepsin the centrifuged precipitate is replaced in the 47.5 ml solution left after sample taking, then with 3 g K_2HPO_4 added and 10 N NaOH the value of the pH is adjusted to 8.4 and 2.5 mg trypsin (4.400 F. N. unit/mg) is dissolved in the solution warmed up to 38 °C. Decomposition with trypsin is likewise carried out in an ultrathermostat at 38 °C with constant shaking for 12 hours. After this a sample of 2.5 ml is again taken for the purpose of analysis.

3. Decomposition with erepsin. After sample taking the centrifuged precipitate is again re-added to the 47.5 ml solution left behind; the pH value of the cloudy liquid is adjusted with H_2SO_4 to 7.8, then 100 mg erepsin (Koch Light Lab. Ltd., Cat. N° 2451h) is dissolved in it, 1—2 drops of toluene added, and decomposition with erepsin carried out for 24 hours in the manner described above. After 24 hours the glass is placed in hot water for 10 minutes (to stop the action of the enzyme), then when the solution has cooled down, its pH value is adjusted with H_2SO_4 to 2. The analyses are performed with solutions from which the precipitate has been removed by centrifuging.

During the decomposition of the analytic material a blind run is also made, using 50 ml 0.1 N HCl and the enzymes.

4. Reference materials for the enzymatic decomposition of analytic substances. To evaluate the examination results a double reference is used: freeze-dried egg-white from hen's eggs and freeze-dried whole-egg protein. Egg-white was found to be the most suitable model for a methodological check-up of enzymatic breakdown, while the whole-egg protein also used gives results serving as a basis for comparison in in vitro decomposition. Fat was removed from the freeze-dried whole-egg protein by a cold procedure, using chloroform to prevent denaturation by heat.

B) *Description of analytical methods*

1. Determination of soluble protein content in the material of examination. Protein is dissolved from the material with a 0.9 per cent NaCl solution at a ratio of 1 : 25, then the nitorgen is determined from the extract.

2. Nitrogen determination after decomposition with pepsin, trypsin and erepsin. From the aliquot of the samples taken nitrogen determination is carried out.

3. Determination of free amino acids present in the solution after decomposition with erepsin. When decomposition has been completed, the undecomposed peptides are removed by ultrafiltration from the aliquot of the solution, then from the ultrafiltrate thus obtained

— only containing low-molecular weight peptides and free amino acids at the most — amino acid determination is performed with an automatic analyser according to Moore and Stein.

4. Pilot examination of the extent of decomposition by biuret and ninhydrin reactions. a) Biuret reaction after decomposition with pepsin, trypsin and erepsin. From 1 ml of the sample taken the biuret determination is performed after Gornall's method (Gornall *et al.* 1949). b) Ninhydrin reaction after decomposition with pepsin, trypsin and erepsin. After each kind of enzymatic decomposition, in 0.01 ml of the solution a quantitative reaction is induced with ninhydrin solution according to Moore—Stein (1954).

5. Examination of the products of decomposition by polyacrylamide-gel electrophoresis after decomposition with pepsin, trypsin and erepsin. The optimum polyacrylamide content of the gel used to study the size distribution of decomposition products is 7.5 per cent after decomposition with pepsin and trypsin, and 7.5 or occasionally 15 per cent after using erepsin for decomposition.

To determine the molecular weight of decomposition products it is advisable to use a gel containing 5—7 per cent polyacrylamide and made with a buffer solution containing sodium-dodecylsulphate, with reference materials of known molecular weight (Dunker—Rueckert 1969).

6. Distribution by molecular weight of decomposition products as studied by gel filtration after decomposition with pepsin, trypsin and erepsin. Chromatography is carried out with 200—500 microgramme samples on Sephadex G-100 superfine and Sephadex G-25 superfine 0.6 mm thin layers, with the well-known technique, using reference materials of known molecular weight.

Discussion

With the recognition of the physiological importance of essential amino acids, and as a result of amino acid determination methods having evolved, a protein evaluation originating from logically inconsequent calculations has been generally introduced in which amino acid quantities found in the egg-protein are used as a basis for determining the numerical nutrition-physiological value of other proteins. In this evaluation if the relative quantity of an amino acid in the protein to be qualified is higher than that of the same amino acid in the egg-protein, even in this case it is only taken as 100 per cent, while quantities of the examination material smaller than 1 per cent are regarded as 1 per cent in order to avoid negative logarithmic values.

In addition to the errors of calculation the amino acid composition of the egg protein in itself did not even prove to be the highest biological value (Kofrányi—Jekat 1964a, 1967, 1969, Kofrányi *et al.* 1970, Müller-Wecker—Kofrányi 1973a). Combinations of various proteins are of higher biological value; the highest of all has been found for a mixture of 35 per cent whole-egg-N and 65 per cent potato-N at 0.37 g protein · kg body weight^{-1} · day^{-1}.

Of Kofrányi's highly important experiments we only refer to two which in themselves are conclusive. Curves representing the minimum protein requirements from a mixture of of whole-egg protein and potato protein supplied in different proportions may be of different values, but their slope is of the same tendency and all have a breaking point at the N ratio of 35—65 per cent (Kofrányi *et al.* 1970). This can also be interpreted in the following way: the minimum quantity of protein requirement (i.e. the value of the curve on the ordinate) depends on the individual, while the value of the breaking point on the abscissa is determined by the quality of the proteins.

As regards the fact that the quality of the proteins is not determined by a calculated amino acid index or chemical score, we refer to the figure obtained for an experimental person with a "whole-egg score" of 0.55 g, i.e. higher than the 0.5 g average of 50 experiments (Müller-Wecker—Kofrányi 1973b), attained with whole-egg protein and bean protein supplied in various proportions (Kofrányi—Jekat 1974a).

Although with the reduction of egg-protein the minimum requirement also decreases to a 36 per cent share of the former, the so-called essential amino acid index increases in proportion to the reduction of egg-protein, while the "chemical score" gives a steeply ascending hyperbolic curve.

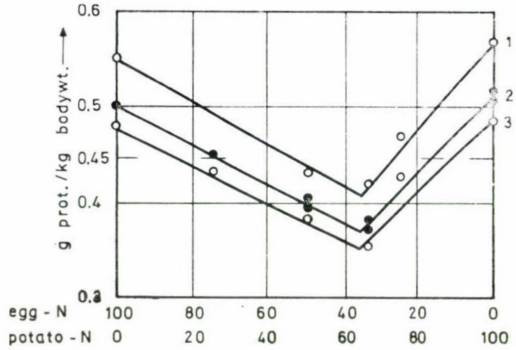

Fig. 1. Minimum requirements from mixtures of whole-egg and potato protein of various ratios (Kofrányi *et al.* 1970). Curves 1 and 3 represent the demands of experimental persons, curve 2 shows standardized average value. On curve 2 the values corresponding to 0.45 g protein · kg body weight^{-1} · day^{-1} are indicated by intersection

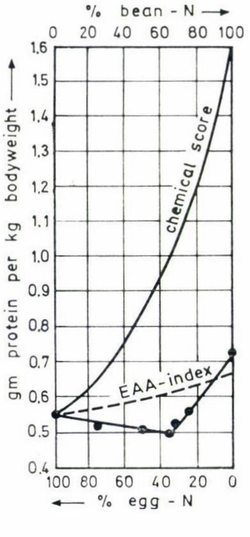

Fig. 2. Minimum requirements from mixtures of whole-egg and bean protein of various ratios and essential amino acid indices and chemical scores corresponding to these compositions (Kofrányi—Jekat 1964a)

The belief in the reality of essential amino acid index and chemical score is shaken to the foundations by the experiment in which amino acid quantities corresponding to various ratio mixtures of whole-egg protein and potato protein were compounded by Kofrányi from synthetic amino acids (**Kofrányi** *et al.* 1970). Results obtained with them confirmed the value pointed out with natural proteins: compared to 0.37 g of natural proteins, from synthetic amino acids 0.382 g · kg body weight^{-1} · day^{-1} was the minimum requirement from an amino acid mixture corresponding to 30 per cent whole-egg protein-N and 70 per cent potato protein-N. (The slight difference can be traced back to methodological faults in the amino acid determination of natural substances, hydrolytic losses occurring in the presence of carbohydrates and calculations on this basis.)

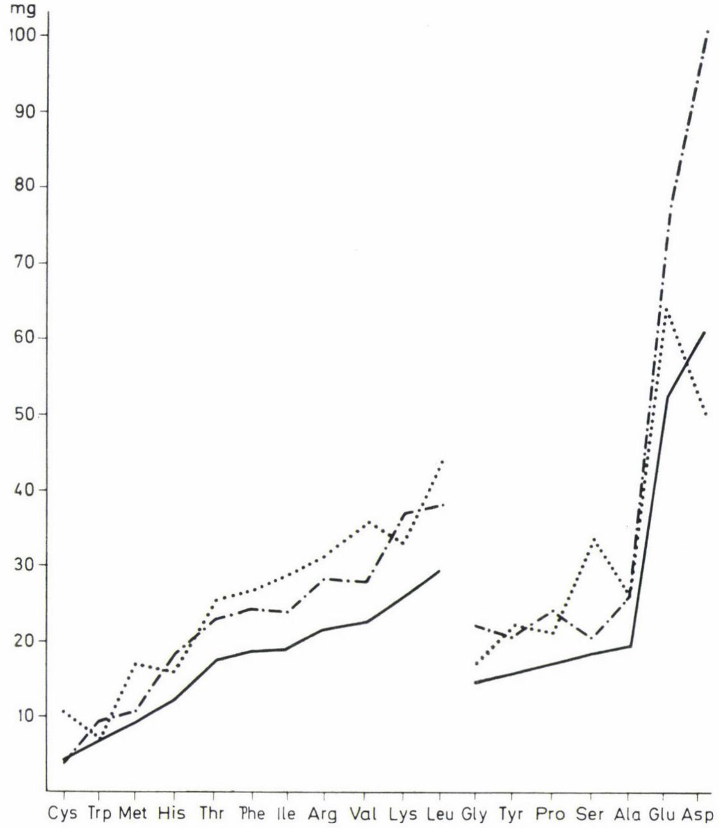

Fig. 3. Amino acid composition in 0.5 g whole-egg protein, 0.51 g potato protein and in a 35 : 65 ratio mixture of 0.37 g whole-egg and potato protein. ...... amino acids of 0.5 g whole-egg protein; —.—.— amino acids of 0.51 g potato protein; ———— amino acids of 0.37 g whole-egg and potato protein (35 : 65)

To decide whether or not the essential amino acids are of limiting character, we used the data calculated by Kofrányi for nitrogen in whole-egg protein and in the potato preparation used in his experiments. In Fig. 1 we calculated the amino acid quantities corresponding to 0.5 g whole-egg protein, 0.51 g potato protein and a 0.37 g mixture of whole-egg protein-N (35%) and potato protein-N (65%), then compared them; the calculated quantities are graphically illustrated in Fig. 3. (To make deviations between the curves clearer, we listed the amino acids in the order of their increasing quantities on 0.37 g of a mixture of whole-egg protein and potato protein.)

Whole-egg protein given by itself as well as potato protein and the quantities chosen here of their 35 : 65 ratio mixture are of equal biological value, since these weights satisfy minimum requirements. Since all amino acids of 0.5 g whole-egg protein are required in larger quantities than those found in a 0.37 g mixture of whole-egg and potato protein of 35 : 65 ratio (and in most csses in mixtures of other ratios too), all essential amino acids of this last curve should be considered limiting. Further, on the basis of curves running side by side, we can conclude that the biological value of biologically equal proteins, or of protein mixtures of different composition, is not decided by the quantity of a certain amino acid (e.g. Lys) but by the proportions that the individual essential amino acids bear to each other. The possibility of different proportions of essential amino acids is clearly seen if we take as a basis for our calculations those quantities of the differently composed mixtures of whole-egg and potato protein whose identical weights ensure the nitrogen balance of the organism. If e.g. in the middle (2) curve of Fig. 1, which represents the average value, we choose the quantity 0.45 g · kg body weight^{-1} · day^{-1} to make calculations with, we find that the quantities and ratios of essential amino acids in a 76 : 24 ratio mixture of whole-egg-N and potato protein-N of 0.45 g weight greatly differ from the composition of a similarly 0.45 g mixture of 14 : 86 ratio, although these identical weights of the two mixtures are of equal biological value. These data prove the possibility of different proportions of the necessary amounts of essential amino acids being of equal biological value, notwithstanding the theory of the limiting character of essential amino acids. The cause of correlation between the essential amino acid ratios and their biological values will be revealed by physiological investigations. Meanwhile, according to our present knowledge we can accept — with respect to humans — as optimum amino acid ratio, that composition, of which the smallest amount is sufficient to satisfy the minimum requirement: the 35 : 65 ratio of essential amino acids in a mixture of whole-egg-N and potato protein-N.

When reviewing the above, we find it impossible that the biological value of a protein or the extent of its utilization in humans or animals could be determined by in vitro experi-

Table 2

Amounts of peptides dissolved after enzymatic in vitro decomposition

	N conversion factor	In the analytic material		Dissolved in 0.9% NaCl		Dissolved after decomposition by pepsin		Dissolved after decomposition by pepsin+trypsin		Dissolved after decomposition by pepsin+trypsin+erepsin	
		N · conversion factor %									
		weight %	rel. %	weight %	rel.* %	weight %	rel.* %	weight %	rel.* %	weight %	rel.* %
Egg-white	6.25	82.5	*100*	79.1	*95.9*	75.5	*91.6*	81.0	*98.2*	81.2	*98.5*
Whole-egg protein (extracted)	6.25	81.0	*100*	55.0	*67.9*	75.0	*92.6*	78.2	*96.5*	78.9	*97.4*
potato (freeze-dried)	6.25	11.5	*100*	8.2	*71.5*	8.6	*74.8*	9.8	*84.9*	10.3	*89.3*
Soya	5.71	39.3	*100*	9.55	*23.3*	23.9	*60.8*	33.8	*86.0*	35.9	*91.3*
Maize	6.25	7.65	*100*	2.5	*32.7*	4.1	*53.6*	4.1	*53.6*	4.8	*62.8*
Meatmeal	6.25	53.1	*100*	13.2	*24.85*	30.0	*56.5*	30.4	*57.25*	37.5	*70.65*
Fishmeal	6.25	61.5	*100*	7.0	*11.4*	13.5	*21.95*	34.5	*56.1*	40.5	*65.9*

* The relative percentage is calculated for the amount of N · conversion factor found in the material.

ments. With in vitro experiments we are confined to obtaining information about the preconditions of protein biosynthesis and decomposition of protein food introduced into the organism. In our opinion the most reliable data will be obtained by the method which shows the highest resemblance to natural conditions, and which analyses the products of decomposition from the most possible aspects. It was on such consideration that we used pepsin, trypsin and erepsin in breaking down food and feed, in accordance with the digestive system of man, and of animals with a simple gastric system. Both in the literature and in feed testing standards we find specifications in which enzymatic decomposition is restricted to pepsin treatment, after which the undissolved part is weighed; these procedures, however, say very little about protein decomposition, since the pepsin that splits before and after Tyr and Phe produces smaller or larger peptides, depending on the position of these two amino acids, of which the latter may not even be fully dissolved. (This can be clearly shown by polyacrylamide-gel electrophoresis too.)

Trypsin treatment after pepsin splits the peptides further in the case of arginyl and lysyl bonds, with the result that the amount of peptides considerably increases in the solution; this can be demonstrated by the increased value of the biuret reaction too.

The extent of protein decomposition can best be judged from the amount of free amino acids obtained after breaking down with erepsin. Although the difference between in vitro and in vivo conditions can be the sharpest here, still a comparison with the amount of free amino acids originating from whole-egg protein can be regarded as a reliable basis for getting information about the extent to which the protein can be broken down, and in qualifying products of the same kind, e.g. in testing various soya meals. (Deproteinization, a necessary preparatory process of free amino acid determination, must definitely be carried out with ultrafiltration.)

The first informative data on the nature of food and feed proteins are obtained from the determination of proteins soluble in 0.9 per cent NaCl. Its quantity — compared to the total proteins — serves, however, as a basis in comparing the effects of enzymatic decompositions, rather than as a parameter replacing decomposition or indicating the possible extent of decomposition. As a result of enzyme treatments the amount of insoluble protein increases gradually (or sometimes suddenly) after each step. Although a higher proportion of insoluble protein indicates a lower value for the examined protein (maize, meatmeal, fishmeal), large amounts of peptide molecules in the solution are far from proving the high utility of food and feed protein. In Table 2 various possibilities of solubility are shown, using data from 7 selected products. (The data of the two reference materials — egg-white and whole-egg protein — presented in the table can be regarded as standard values from which any deviation larger than ±1% suggests methodological faults. The results of the 5 food and feed proteins originate from the analysis of one sample each; data characterizing the different food- and feedstuffs can only be established and standardized after processing a large number of samples from different origins.

With the ninhydrin colour reaction of α-amino groups the increase in the number of peptides and free amino acids in consequence of enzymatic decomposition can be followed. Since the result of the biuret reaction is inversely influenced by the number and size of the peptides — if peptides smaller than tetrapeptides are formed —, therefore the value of the biuret reaction decreases after decomposition with trypsin, while the result of the ninhydrin reaction increases step by step. The results of the biuret and ninhydrin reactions, and the pictures given by polyacrylamide gel electrophoresis and thin layer gel filtration are in agreement, and typical of each food or feed protein.

Reduced biuret reaction will not be obtained after decomposition with trypsin in the case of materials where, beside the protein, a trypsin inhibitor is present (e.g. soybean); therefore, the result of decomposition by trypsin gives full information about the efficiency of a preliminary procedure applied to inactivate the inhibitor (e.g. toasting of soybean). With a lysyl bond trypsin splitting will be inhibited even if the ε-amino group of Lys is blocked (e.g. by carbohydrates in consequence of heat treatment; if the enzyme-resistant lysyl bond is not split, not only the further decomposition but also the utilization of the peptide will be inhibited). The amounts of free and blocked Lys-containing ε-amino groups in the protein can be determined by Carpenter's method or by one of its modified forms. It must be noted, however, that the determination of ε-DNP-Lys quantities is not enough in itself from which to draw conclusions on the prospective percentage utilization of protein, since it has many other causes besides the free amino group of Lys. Consequently, we should be careful both in evaluating the result of dinitrophenylation and in choosing the occasion when the application of the procedure is justified. Data on this subject are provided on the basis of a large experimental material by Erbersdobler's works (BRÜGGEMANN—ERBERSDOBLER 1968a, 1968b, ERBERSDOBLER *et al.* 1968, ERBERSDOBLER—DÜMMER 1971).

Table 3

Results of enzymatic in vitro decomposition of hen eggwhite and whole-egg protein

	Egg-white	Whole-egg protein
Total N. 6.25	82.5%	81.0%
N. 6.25 soluble in 0.9 per cent NaCl	79.1%	55.0%
Biuret reaction, E from 10 mg N. 6.25	0.104	0.096
Ninhydrin reaction, E from 1 mg N. 6.25	0.158	0.305
After decomposition by pepsin:		
Dissolved N. 6.25	75.5%	75.0%
Biuret reaction, E (from 10 mg)	0.332	0.407
Ninhydrin reaction, E (from 1 mg)	0.567	1.086
After decomposition by trypsin:		
Dissolved N. 6.25	81.0%	78.0%
Biuret reaction, E (from 10 mg)	0.321	0.326
Ninhydrin reaction, E (from 1 mg)	0.926	1.390
After decomposition by erepsin:		
Dissolved N. 6.25	81.2%	78.9%
Biuret reaction, E (from 10 mg)	0.289	0.306
Ninhydrin reaction, E (from 1 mg)	1.235	2.190

Analytical data, summed up in Table 3, on the decomposition of egg-white and whole-egg protein, which are used as reference materials, serve as the basis for the decomposition studies.

On the basis of an amino acid determination carried out after decomposition by erepsin, 41.5 per cent of the total N. 6.25 was found in the form of free amino acid in the solution. It is surprising that with the exception of Pro the relative quantities of all amino acids are approximately proportionate to those composing the native whole-egg protein. This, on the one hand, raises the known problem of whether Pro-peptides are decomposable at all, and on the other hand, points to the cause of the reduced biological value of collagen and collagen derivatives.

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CONTRIBUTIONS

to the paper of P. Greguss: "Dichtotomous branching of vascular bundles in the stem and leaf of maize and their phylogenetic importance" published in this periodical, **24** (3—4).

IS THE BRANCHING OF VASCULAR BUNDLES IN THE STEM AND LEAF OF MAIZE DICHOTOMOUS?

The view expressed by Greguss that divergence of monocotyledons and dicotyledons occurred apparently early in the process of evolution of the Angiospermae, has also been expressed by several other authors (c.f. Moore—Uhl 1973). I find it, however, difficult to accept Greguss's view that the vascular bundles in the stem and leaf of maize branch dichotomously. From morphogenetic studies it has been well established that the procambial strands, from which the vascular bundles develop, differentiate below the position where leaf primordia are formed on the shoot apex. We may, therefore, regard all vascular bundles of the stem of the higher plants as leaf traces; leaf bundles of upper nodes join leaf traces of lower nodes. The level of their fusion differs in the different plant species.

The data of Volcsánszky as presented by Greguss show that the number of vascular bundles in the maize stem is smaller in the lowermost and uppermost internodes than in the intermediate ones. This is a result of a smaller number of leaf bundles entering the lowermost and uppermost nodes. The lowest and uppermost leaves of the maize shoot are usually narrower and probably have less vascular bundles than the rest of the leaves.

Zimmermann—Tomlinson (1965, 1966) suggest that in palms all bundles maintain their individuality throughout the stem, and each of these vertical bundles give off leaf traces. This interpretation suggests that in the palm stem the bundle branching is monopodial. Accepting the view that all the primary vascular tissue in the stem of the higher plants was reduced to such an extent that it forms only a network of extended leaf traces (e.g. in most dicotyledons) or many separate longitudinal systems of leaf traces with cross-bridges in the nodal regions (e.g. in monocotyledons), I would rather interpret the branching of the palm stem bundles as sympodial, each bundles ending in a leaf. The facts as given by Greguss, according to which in the maize stem about 50 per cent of the vascular bundles of each node enter a leaf and about 50 per cent continue in each subsequent upper internode, seem, to me, to confirm the view that here also the branching of the vascular bundles is sympodial and not dichotomous.

Another point I would like to mention is that if the branching of the vascular bundles in the maize stem were dichotomous, I would expect the branches entering the leaves to divide there too in a dichotomous manner. This, however, as far as I know, does not occur.

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IS IT FROM THE STALK TO THE LEAVES OR FROM THE LEAVES TO THE STALK THAT THE VASCULAR BUNDLES RUN IN THE MAIZE PLANT?

On the basis of Erzsébet Simon-Volcsánszky's paper Professor Pál Greguss compiled interesting analysing remarks. He considers Mrs. Simon's published material suitable to prove by the supposedly dichotomous vascular bundles of the maize the phylogenetic relationship of Monocotyledonopsidae with the similarly dichotomous Thallophytae and the more ancient Cormophytae. He says that "the dichotomy of vascular bundles has to be valued as a kind of phylogenetic character and relic phenomenon in the maize stalk too".

In this context only two questions may be raised but they are very important when evaluating the statement. One of the questions is whether the vascular bundles pass out of the maize stalk to the leaves or arrive from the leaves in the stalk. The other question is whether or not it is a case of pseudodichotomy which — while existing at an early stage of development — later becomes indistinct. To decide the question would require the thorough analysis of the early stage.

As regards the first question, it can be established that if the vascular bundles come from the leaves then it is joining, growing together rather than branching off that we must speak of. This is the very opposite of dichotomy and only resembles it in a negative sense. Since in the maize stalk leaf trace bundles are found we should speak of the bundles as joining rather than branching off. The author herself writes toward the end of her paper that Zimmermann and Tomlinson as well as earlier Desfontaines and von Mohl speak of the vascular bundles as descending and not as "Ascending" in the monocotyledons. The highly valuable dissertation is seen thus in a different light and further evidences are required to prove the phylogenetic thesis.

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HOW DID THE MONOCOTYLEDONS ORIGINATE?

In her dissertation "Effect of some factors on the anatomy of the vegetative organs of the maize stem" (1973), S. VOLCSÁNSZKY E. dealt, among other things, with the determination of the number of vascular bundles in the stem and leaves and traced changes occurring in individual internodes from the tip to the base of the shoot. She reached the conclusion that twin bundles frequently occur at all cross-sectional levels of the internodes and grow together vertically.

Greguss first remarks quite correctly that if she had examined the stem from the base to the tip she would have found that adjacent bundles separate. This observation seems to me to be of great importance, because even in many textbooks there is no clear representation of the actual relationship between the course of the vascular bundles in the stem of monocotyledons. It is often said, for example, that numerous bundles enter the stem from the leaves and fuse with other bundles during their downward course; but there can be no talk of fusion. For the determination of the upward dichotomous branching of the vascular bundles, and consequently their differentiation too, takes place just under the vegetative node. Thus the bundles do not enter the stem from the leaves, but enter the leaves from the stem.

Greguss also gives this dichotomous branching a considerable phylogenetic significance. On the basis of evaluations made by Volcsánszky on the number of vascular bundles in the individual internodes, starting from the lowest internode and proceeding towards the tip, Greguss comes to the conclusion that the ratio of the bundles branching off into the leaves is always about 50%, which speaks in favour of the dichotomous branching of the bundles in the nodes. A similar dichotomous branching has been observed by Zimmermann—Tomlinson (1965, 1972) in other kinds of monocotyledonous plants.

Finally, Greguss gives a detailed discussion of the phylogenetic significance of this dichotomy. Starting from the fact that dichotomy is present even in the simplest filamentous algae, continues up to the angiosperms and, as has been shown, can also be found in the vascular bundles of maize, a monocotyledon, it can, to a certain extent, be considered as a relic of this development and thus as a phylogenetic character.

From these results and from the fact that in the dicotyledons completely different types of branching are found, which are also anchored in the developmental history of the plant kingdom, it may be concluded that, due to their tendency to dichotomy, the monocotyledons cannot have originated from the monopodial dicotyledons, but must have developed independently. Thus, a polyphyletic rather than a monophyletic development may be postulated for the angiosperms. The question of the phylogenetic development of the monocotyledons has consequently been reopened and simultaneously it has become a matter for discussion, whether their frequently postulated origin from the dicotyledonous Polycarpicae, which is based on certain parallels observed between them, is correct or not.

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IS THE SEPARATION OF A VASCULAR BUNDLE EQUIVALENT TO THE TRUE DICHOTOMIC BRANCHING OF THE MAIN AXIS?

On the basis of the data of a candidate's thesis (Volcsánszky 1973) professor Greguss claims that the vascular bundles of the maize stem branch off dichotomously at the nodes, one branch running upward in the stem, the other turning outward into the leaf. From this he draws the conclusion that — since in dicotyledonous plants there is no dichotomy — the monocotyledons could not have originated from the dicotyledons, but attained their present stage of development independently. This is the main point of the paper.

The question is far from being as simple as this. The development of the vascular system in the shoot apex is closely linked with that of the leaves, both forming a single coherent process. Many questions remain to be cleared up here.

For example, it is still under discussion whether the vascular system as a whole is of procambial origin, or the so-called "residual meristems" have a part in its development too. Another undecided question is whether the cell divisions leading to the formation of the first conducting elements continue downwards from the leaf primordium to reach the conducting system of the axis, or upwards, from the axis toward the leaf primordium.

The orientation is particularly difficult in the stems of monocotyledons like *Zea mays*, where the arched descent of the leaf trace bundles, the complicated structure of the nodes make it extremely difficult to find the junction of the leaf traces. From the data of MERICLE (1950), SHARMAN (1942, 1945) and ESAU (1969) it is known that in the stem of *Zea mays* the stronger and older trace bundles differentiate acropetally, while the weaker ones start from the node and grow downwards in the axis and upwards in the leaf.

In the leaf primordium the procambium — from which the bundles are formed — differentiates from the subdermatogen initials. These bundles often pass through a number of internodes before joining the trace bundles that start from the upper leaves and descend in the stem. The situation is made even more complicated by the fact that with the advance in age of the leaves the number of leaf trace bundles may increase or even multiply. There are literary data — though not sufficiently convincing ones (a remark by ESAU 1969) — on the existence of side bundles which are in no connection with the lateral organs.

It is well-known that the larger bundles of the leaf sheath of *Zea mays* entering the stem through the node tend inward, while the smaller ones remain in the peripheral part of the stem. The medial bundle runs to the centre of the stem, the other ones take a position between the centre and the periphery. Thus, in the cross section of an internode the inner bundles partly consist of the trace bundles of the nearest leaf, partly are composed of the side bundles of the upper internodes, while the bundle circle consisting of the peripheral and small bundles is mostly formed by the thin trace bundles of the leaf immediately above.

Another difficulty is that the bundles pass through the node slantwise or horizontally, and in this position separate and join again while their original number also changes.

Under these conditions we cannot speak of the bundles simply dividing into two in the nodes, half of them continuing its way in the upper internode while the other half in the leaf. The numerical data do not confirm this either, except for the 8th internode seen in the table, and even there no data are available concerning the actual number of bundles in the leaf!

The separation of a bundle — if they really do separate and not join — is not equivalent to the genuine dichotomous branching of the main axis. And I do not agree with the phylogenetic conclusions drawn from the "dichotomous branching" of bundles, and do not accept it as an argument against the possibility of monocotyledons having originated from dicotyledons. This single character is not sufficient to refuse the generally held phylogenetic conception, all the less so because it is well-known that the vascular bundles of some dicotyledonous stems bifurcate above the trace bundle descending toward the lateral organ, and join in that way the two adjacent bundles. By analogy this too can be regarded as "dichotomy", and then the author's otherwise very interesting and remarkable phylogenetic conception is shaken to its foundation.

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CAN THE VASCULAR SYMPODIA BE CONSIDERED AS A SERIES OF DICHOTOMIES?

In a paper in Phytomorphology *18* (1968), Greguss extends his ideas on the phylogeny of vascular plants to the Angiosperms. According to him the Dicotyledons and Monocotyledons originate as two separate lines right from the start of land plants. The argument was that the monopodial and the dichotomous manner of branching originate quite independently of each other. And whereas in the Dicots no dichotomous branching occurs, in Monocots dichotomous branching occurs frequently. Greguss mentioned dichotomously branching stems of *Dracaena*, *Pandanus*, *Yucca* and *Hyphaene*.

Greguss' paper of 1968 was challenged as to the facts by Tomlinson—Zimmermann—Simpson in Phytomorphology *20*, 1970 (not cited by Greguss in his present paper). In *Aloe*, *Cordyline*, *Dracaena*, *Pandanus* and *Yucca*, all of which have forking stems, it is known that the vegetative apex is transformed completely into a terminal inflorescence. After flowering, two lateral buds of equal potential grow out below the remains of the inflorescence. The result is a forking sympodium. Only in Palms dichotomy almost certainly occurs, viz. in the stems of Hyphaene (as disclosed by Schoute in Rec. Trav. Bor. Néerl *6*, 1909), and the rhizomes of *Hypa* (Tomlinson in: Ann. Bot., *35*, 1971). Fisher has recently described dichotomy in the stems of *Chamaedorea* (Amer. J. Bot., *61*, 1974).

As regards the present paper by Greguss on the vascular bundle course in the stems of Zea mays, it must be regretted that Greguss again brings forward dichotomous forking in *Aloe*, *Dracaena*, *Pandanus*, and *Yucca*, ignoring the facts, as these were once more depicted by Tomlinson—Zimmermann—Simpson. Judging by the description, the stem of *Zea mays* contains a huge number of discrete vascular bundles, each giving off a branch toward a leaf at each node. Precisely these continuous primary axial vascular bundles giving off leaf traces at successive nodes have become known in recent times to characterize the stems of many Angiosperms, Gymnosperms and Progymnosperms. They are considered distinct vascular sympodia, and can be parastichous or orthostichous (Balfour—Philipson in: Phytomorphology *12*, 1962; Namboodiri—Beck in: Amer. J. Bot. *55*, 1968; Slade in: New Phytol. *70*, 1971; Devadas—Beck in: Amer. J. Bot. *59*, 1972). Whether or not the vascular sympodia can be considered as a series of dichotomies is a question very difficult to answer (see Bock in Geol. Center Res. Series, North Wales, Pennsylvania, *2*, 1962). The work of Zimmermann—Tomlinson (Adv. in Bot. Res. *3*, 1970, and Bot. Gaz. *133*, 1972) shows that also in Monocots continuous helicoid primary vascular main bundles exist in the stems. The findings of Greguss in Maize, as derived from the work of Volcsánszky, are in accordance. However, it is clear that, as the vascular sympodia occur in Dicots as well as in Monocots, no argument can be found in favour of two different lines so widely separated for Dicots and Monocots. Also the flower structure speaks definitely against a very wide separation, especially as regards the ovule and the stamen structure, the gametophyte reduction, and the double fertilization resulting in the secondary endosperm.

Finally, dichotomies in the ovules and the stamens occur in Dicotyledons as well as in the Monocotyledons. I may refer the reader to my paper on dichotomy in Proc. Kon. Nederl. Akad. Wet. C, 77, 4, 1974.

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SHOULD WE START FROM THE ROOT OR FROM THE TASSEL WHEN EXAMINING THE MAIZE PLANT?

In No. **24** (3–4) of Acta Agronomica Hung. Greguss thoroughly discussed some anatomical observations in the candidate's dissertation of S. Volcsánszky E. From Volcsánszky's work ("Effects of some ecological factors on the anatomy of vegetative organs in the maize stalk"), he analyses in detail the changes in the number of vascular bundles in the stalk, particularly from a phylogenetic point of view. The paper introducing a discussion encompasses vast areas of botany, and with references to Volcsánszky's work touches upon descriptive and analytical anatomy (dichotomy of vascular bundles), evolution of monocotyledons and their phylogenetic relations, etc. Of the great many problems raised I should like to deal with a single question: where should we start looking at the plant from?

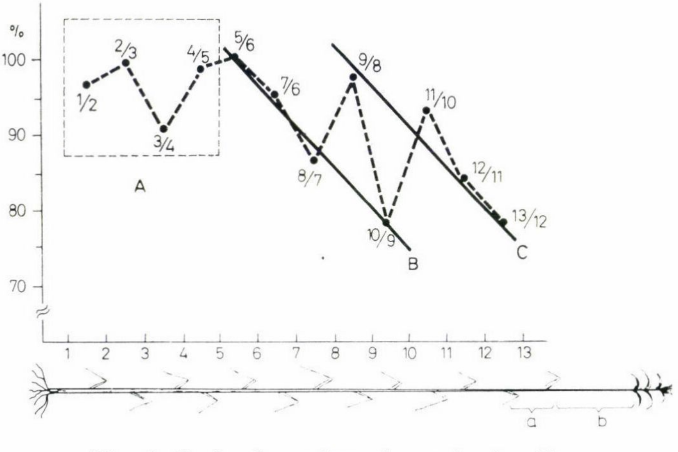

Fig. 1. Ratio of number of vascular bundles

First we have to answer some minor questions:

1. It is not clear from the citations whether in Volcsánszky's data internode I is — sensu stricto — the part of the stalk between the two uppermost leaves (Fig. 1a), or the lower section of the floral axis (Fig. 1b) is — s. lato — included too.

2. It remains to be decided how much the ecological factors modify the genetically determined morphological gradients.

Now we have arrived at the question raised before: from what direction should we look at the plant?

Table 1

A comparison between the increase in the number of vascular bundles at the individual internodes and the standard of the plant

Obtained values					Theoretical values			
a	b	c	d	e	f	g	d′	e′
	0							
13	1		385	0.777	100	0.87	90	0.1818
		0.967						
12	2		398	0.804	150	2.4	100	0.2020
		0.992						
11	3		401	0.810	200	3.6	150	0.3030
		0.907						
10	4		442	0.893	300	5.7	200	0.4040
		0.980						
9	5		451	0.911	400	7.8	250	0.5050
	$\boxed{5.5}$	1.000	$\boxed{495}$	$\boxed{1.000}$				
8	6		451	0.911	450	8.8	300	0.6060
		0.951						
7	7		429	0.866			350	0.7070
		0.869						
6	8		373	0.753			400	0.8080
		0.976						
5	9		364	0.735			450	0.9090
		0.777						
4	10		283	0.572				
		0.933						
3	11		264	0.539				
		0.841						
2	12		222	0.448				
		0.779						
1	13		173	0.349				

Obtained values. a = serial number of internodes according to (Volcsánszky—Greguss; and b = according to Maácz; c = ratio of the number of vascular bundles in two adjacent internodes; d = number of vascular bundles on the side of the root; e = th-ratio of vascular bundles = d/495.

Theoretical values. From the equation of the hyperbola in Fig. 2, if f = x = number of vascular bundles; g = y = serial number of internodes (the A equation and its co-ordinates). The hyperbola of Fig. 3, if d' = y = number of vascular bundles, then e' = position of internodes (x) and the number of vascular bundles (y) according to the unit scale.

Volcsánszky's data proceed from the tassel towards the root. Greguss (though maintaining the original numbering) thinks it better to start from the root. He finally settles the question by declaring that, after all, it does not make any difference from the point of view of his calculations, as the place (internode) is in both ways defined. His calculations are based on the assumption that, in theory, twice as many bundles ($b = 2a$) can be found in the node as in the internodes (a). Namely, half of the bundles in the node bend out into the leaf sheath ($c = b - a$), that is

$$e\% = \frac{c}{b} = \frac{b-a}{2a} = \frac{2a-a}{2a} = \frac{2a}{2a} - \frac{a}{2a} = 1 - \frac{1}{2} = 0.5 = 50\% .$$

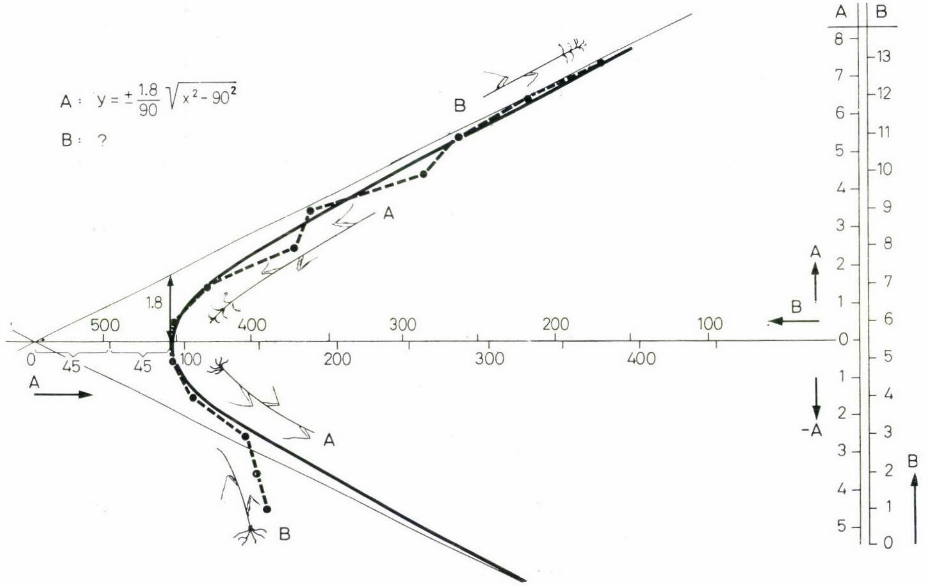

Fig. 2. Numbering of the internodes towards the tassel and towards the roots

Without touching upon the phylogenetic problems arising here, the following questions ought to be answered:

1. From what direction does the plant count its vascular bundles, that is, what is the gradation of the bundles? (Phytocentric viewpoint.)

2. What programme is realized in the number of vascular bundles in the course of ontogenesis?

3. Finally: from what direction should we examine the plant to obtain the widest possible information? (Reconciliation of the phytocentric and antropocentric viewpoints).

Further on we shall try to answer the above questions without any hypothesis and equalizing calculations, relying exclusively on Greguss—Volcsánszky data. Equalization must have been carried out by the plant itself, otherwise it would not have survived while proceeding from the vegetative to the generative phase. (The calculations are of limited exactness; further on, the internodes will be numbered from the root; root = internode 0.)

It can be seen at a glance that the number of vascular bundles increases up to internode 5 or 6, then decreases (Table 1. d.). Their percentage ratio (c. 100) between the internodes (Table 1. c.) is shown in Fig. 1. The numerical ratio of the vascular bundles changes according to definite rules, the directional tangent of parallels B and C is -5.5, the A-set is "seemingly" unregulated.

In internodes 5 and 6 the number of vascular bundles is identical, from here towards both the apex and the root it decreases. Let us number the internodes from here (internode "5.5"=0) towards the tassel and the root, respectively, (Y-axis), and the vascular bundles on the X-axis in the opposite direction. We obtain a surprisingly regular hyperbola (B). If we want to state its central equation the origin can be easily determined with the aid of the asymptotes, but in this case numbering must be started from here (A) (Fig. 2).

The model — though a little complicated — characterizes a system (the whole plant), but it is exactly the two-way coordinates that throw light upon the main question: from what direction should we consider the plant?

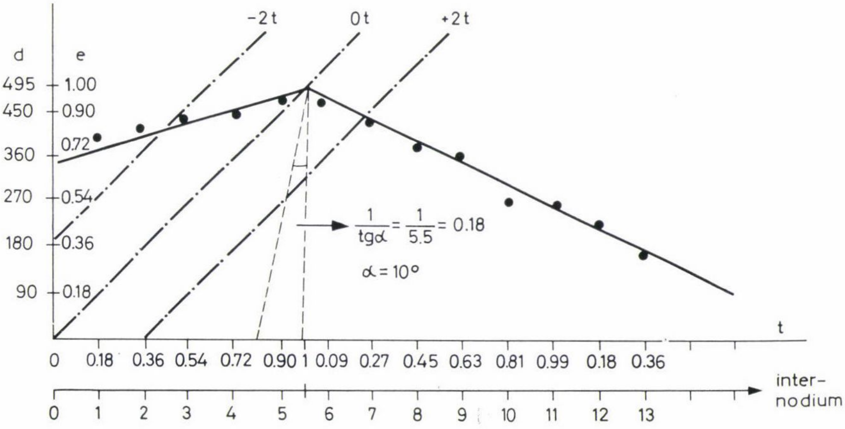

Fig. 3. Depiction of time in the right-angled system of coordinates

If it is done from the original direction (B-abscissa), then the result will be: the plant counts backwards, its counting apparatus is between internodes 5 and 6 (= node), and the control is linked with a genetically fixed value. In this case environment has lost its importance.

If we decide on the basis of the central equation of the hyperbola (A-abscissa), then we sit in a virtual origin (probably in the place of the female flower, there is no reference to this in the table), whose value is about 495 in the B-system. In the A-system the vertex of the hyperbola is at a distance of 90 units from the origin, the plant is "torn" in two, because the lowest values are nearest to us.

The kinetic view of morphology, the approximation to the limit value are the same in both cases, but the model is

a) too complicated, dual,

b) the plant is isolated from the surroundings,

c) the estimation of the upper limit (453) is rendered possible by the B-model, but the lower limit is not defined by it. The equation of the A-model gives an estimate (cca. 90) for the lower limit but may set the upper one at infinity.

Further discussion of the question — after we have put the system into motion — will require a chronometer. In place of a chronometer periodical phenomena can be used. We use the internodes as a chronometer, and the "time" while the number of vascular bundles runs in 5.5 "ticks" over a series of "unarranged" numbers from 0 to 495 as unit. Thus 5.5 = unit t, 495 = unit y (Fig. 3). 1 t "minute" consists of 5.5-times $1/5.5=0.1818$ "second" steps. By this the abscissa is calibrated. We adjust the ordinate to the same measure. By this we have ensured that the rate of change of the number of vascular bundles corresponds to the clock of the plant (5.5 was the directional factor of the lines B and C in Fig. 1).

Now we can compare the increase of the number of vascular bundles in the individual internodes to the standard of the plant (Table 1), e.g. 385/495 = 0.777. It can be seen that the number of vascular bundles is approximately 91 per cent (in the central equation of Fig. 2 it is 90) this "virtual" but not hypothetic number (= 495). The value of *th* (tangens hyperbolicus), that of *ch* (cosinus hyperbolicus) and the pertinent x-values can be found in the e-column hyperbolic function table.

The *th* can be interpreted as acceleration related to a standard of unit velocity. To stick to the former example

$$\frac{495-385}{495}=\frac{495}{495}-\frac{385}{495}=1-th\ .$$

With such calculations the most frequently used formula is

$$\frac{1}{\sqrt{1-th^2}}=\frac{1}{\sqrt{1-\left(\frac{385}{495}\right)^2}}=ch,\quad \text{resp.}\ \frac{1}{\sqrt{1-\frac{v^2}{c^2}}}$$

in the form known from the theory of relativity.

Examining Fig. 3, we find an excellent agreement without any equalization.

In the rectangular system of co-ordinates of our laboratory time passes evenly (*t*-axis of equal scale). On the other hand, on the sides of the hyperbola the scale is longer than its projection on the abscissa ("time dilatation" or change of the lengths of internodes, respectively).

The co-ordinate axes of the plant show a high deviation relative to ours: we see an accelerating system which slows down around internodes 5 and 6, then gathers speed again; just as if we saw two plants .And in fact, there are two of them; female and male parts have different ranges of temperature, and elongation too takes place in two main phases. At the sides of the hyperbola "acceleration" (increase in the number of vascular bundles) is 1/5.5 = = 0.18 = 18 per cent.

The problem of time: the number of bundles in internode 2 and 7 are rather close to one another. From point 7 the plant sees as if its position corresponded to that in internode 2, and sees point 2 on our time scale to be *−2*. The identical numbers are not of the same value. By the way: the "laboratory" time-axis of fractional sowing is continuous, the synchronization of the hours is possible, while in the case of a yearly repetition this is impossible and promises precious little genetic use.

Agreement with the calculated values is remarkable on the side of the tassel, while on the side of the root the rise of the points of measuring is very low and only begins to increase in internodes 3 and 4: in an environment inadequate to its genetic properties the initial development of this maize plant was slow. Between points 9 and 10 the system accelerated, the tangent of internode 10 is steeper, between points 9 and 10 the distance is greater, but point 11 suggests a slower than average development.

Where does our diagram end? Upwards on the ordinate the minus-times increase. The side towards the root seems to be much longer (slow differentiation), until reaching the X-axis, that is the *0* vascular bundle. This is the seed stage, or more precisely the not yet fully differentiated embryo. Towards the tassel the question is morphologically nonsensical.

The conclusion of the analysis: with the proper co-ordinates (suited to the plant!) a single datum (in present case 495) may be sufficient. All living creatures are capable — within the existing life conditions — of compensating by themselves the discrepancies arising in their environment by a series of constant feedbacks, and realizing their own genetic programmes by maintaining a constant homœstatis.

*

The cross section of the stalk of Zea, the closed vascular bundle, has been for more than a hundred years — and will certainly be for centuries ahead — part of the curriculum for first-year students at the universities of the world.

I dedicate — though a little late — this work to my former professor, the 85-year-old Pál Greguss.

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DOES DICHOTOMY FREQUENTLY OCCUR AS AN ATAVISTIC FEATURE IN ANGIOSPERMOUS PLANTS?

The dichotomy of vascular bundles can be observed not only in the stem of the angiospermous plants, at the points where the leaves depart from the stem, but — if the route of the vascular bundles is followed — very often in the leaves too.

The pattern of veins in the leaf of *Kingdonia uniflora* (family: *Ranunculaceae)* represents the most ancient type. Starting from the petiole the leaf veins branch off repeatedly at sharp angles. The leaf is triangular, the leaf apex unevenly serrated.

According to our own observations, different stages of dichotomy may be discerned in the vascular system of the leaves.

In one type the blades of the leaflets forming the compound leaf show an extreme stage of division. Below the segments the main rib of the leaflet bifurcates and is continued in two equal lateral veins, while the blade of the leaflet is also divided into two *(Helleborus).*

In another type the leaf is only slightly divided, the leaves are broad egg-shaped, circular or kidney-shaped. In the middle of the leaf blade lateral veins starting from the main rib are often divided into two equal secondary laterals. Sometimes they even continue to bifurcate, thus reaching the edges of the leaf, e.g. in the leaves of *Bergenia crassifolia.* If the leaf is circular or kidney-shaped the veins running radially bifurcate once half-way, then again before reaching the edge of the leaf. (This can be seen in the leaves of *Nuphar luteum,* as well as in the leaves of several *Ranunculus* species e.g. *Ranunculus ficarian, R. flabellifolius* and *R. cassubicus.*)

A fine example of repeated vein dichotomy can be seen in the leaves of *Asarum europaeum.* The radial primary veins of the kidney-shaped leaves bifurcate before they are even halfway across the leaf blade, and, by repeating this three or four times, cover the entire leaf surface with a network.

In the third type of dichotomy the veins bifurcate near to the edges of the leaf, while closer to the main rib they branch off monopodially. Such type of dichotomy at the edges of the leaf can be seen in the pinnate nervure of *Cerasus avium,* and also in the leaves of *Viola odorata* and *Hedera helix.* In many cases injured *Hedera* leaves divide into two equal parts at the apex, in the form of a two-pronged fork. The form of circular and kidney-shaped leaves is, in fact, the result of dichotomy. Dichotomy causes the uniform growth of the leaf edges, and the development of the leaf blade to equal distances from the petiole. It is the consequence of the uniform development of bifurcate veins.

Dichotomy can be observed not only in leaves but also in shoots which develop like leaves. For example, the cladodia of *Zygocactus truncatus* divide in many cases into two flat branches, then continue to grow in two flat lateral axes each, though sometimes three lateral axes develop.

Dichotomy can be observed during the development of the reproductive organs too. The nervure of the petals, for instance, shows a dichotomous pattern in many plant species, e.g. in *Prunus spinosa.*

Primordial dichotomy is shown by the stamen. At the end of each dichotomus filament an anther develops in the flowers of *Carpinus betulus, Tilia ulmifolia* and *Corylus avellana.* The male flowers of *Ricinus communis* give a fine example of dichotomy. The bush-like male inflorescence shows a dichotomous pattern.

In summary it may be said that dichotomy often occurs in angiosperms as an atavistic feature. There are cases where branching is based on pure dichotomy, while in other cases monopodial and dichotomous branching occur simultaneously, as in some of the examples listed.

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RECENSIONES

Dimény Imre

A GÉPESÍTÉS-FEJLESZTÉS ÖKONÓMIÁJA A MEZŐGAZDASÁGBAN

I. Dimény: *A gépesítés-fejlesztés ökonómiája a mezőgazdaságban* (Economy of mechanization development in agriculture). Akadémiai Kiadó, Budapest, 497.

Countries where mechanization becomes a general factor of agricultural development in the 1970's are certainly lucky. This can be emphasized first of all because the biological factors of production make it possible to create or maintain an economic balance. The application of potential technical and biological factors requires, naturally, appropriate forms of enterprise and management.

Today Hungary can rightly be placed among the countries where all the conditions of agricultural mechanization are provided or where they even form a genuine part of the economic balance of management. However, the purchase of machines and the uniform level of the other production factors only offer the possibility of balanced management. Agricultural development based on the exploitation of the possibilities of mechanization is justified only by the economic efficiency of application.

The publication of this summarizing work was therefore important and opportune.

The 497-page book was published in 1975 by the Publishing House of the Academy of Sciences, Budapest. The author has divided his book into four parts. Part I is entitled: Economic concepts applied to mechanization (pages 15—109). The title of Part II is: Methods and possibilities of mechanization development (pages 109—170). Part III discusses the agricultural and horticultural machine and production systems (pages 171—333). Finally, Part IV deals with the machine and production systems of livestock farming (pages 333—497).

The title of *Part I* is not in full accordance with the content, since the range of subjects discussed here is wider.

Besides the fundamental economic concepts and considerations, technical development as a whole and its major components are discussed here (land and amelioration, biological factors, chemical means and methods, as well as technical factors of development).

To fulfil a natural demand, the author

makes it clear what economic grounds his way of thinking is based on, and the methods by which he has arrived at his conclusions. The wish to be brief and keep to the point may be the reason why some formulations or methodological considerations are too stiff, e.g. in the efficiency calculation on pages 23 and 24 the distribution of yield surplus between fertilization and mechanization; on page 31 the emphasis laid on profitability as one of the factors of economic efficiency. The main point in thinking about mechanization in an economic way is that the consideration of production and development as a whole has the priority; the separate examination of problems can provide a basis only for partial judgements.

In a complete economic consideration mechanization forms part of an overall technical development. The author emphasizes that technical development also involves the inseparable unity of objective and personal conditions.

Part II can be said to be of outstanding importance. It points out the characteristic phases of mechanization in Hungary in relation to the large-scale development of agriculture. It offers an objective evaluation of foreign experiences. It declares that both now and in the future the enterprise should be regarded as a system, within which a place must be created for mechanization. It emphasizes that the vertical and horizontal machine systems should be developed within the enterprises in co-ordination with each other. The quick and efficient establishment of machine systems within the enterprises is promoted by the widely introduced crop-growing and livestock-breeding systems.

Special emphasis is laid in the book on the human factors of mechanization development. The reduced labour force must be made up to full strength, working conditions must be improved and the seasonal nature of the work must be moderated. Mechanization will, however, only be efficient or, indeed, realized at all, if the professional and cultural level of the agricultural workers is considerably improved by continued education and training.

Part III of the book is — understandably — of quite considerable extent, as it is here that the machine systems of field and horticultural production are discussed and a lot of practical experience presented.

First, the horizontal machine systems are discussed in detail (machines for soil cultivation, fertilization, plant protection, irrigation, material transportation, drying and storage). Then the full technology of field and horticultural crops is dealt with, together with their machine systems. Finally, a survey and numerical evaluation is given for the production systems (wheat, rice, maize, sugar-beet, alfalfa, sunflower, soybean, tomato, onion, greenhouse production, peach, grapes) currently known and practised in Hungary.

The text is illustrated by date and figures for the technologies and related machine systems, as well as for the production systems; a basis is provided for adapted calculations at an enterprise level. It is stressed that further development and changes are expected; that is, there is no final solution. The complex view of an individual branch and its integration with the enterprise system will, however, save anybody from considering things isolated from their surroundings.

It is useful that the book gives the essential elements and data of the individual production systems. These data are, however, difficult to handle and evaluate because indices of quite different character and content are presented, mostly without any explanation.

The most difficult task was undertaken by the author when writing *Part IV*. Conceptions and sectoral solutions that can be generalized in livestock breeding cannot, with the possible exception of poultry production, be summed up. The most important factors of production cannot even be synchronized; because of deficient knowledge, technical faults and the very large investment. This means, in fact, that a definite system requires a considerable length of time to be fully developed and efficiently operated even in a single farm.

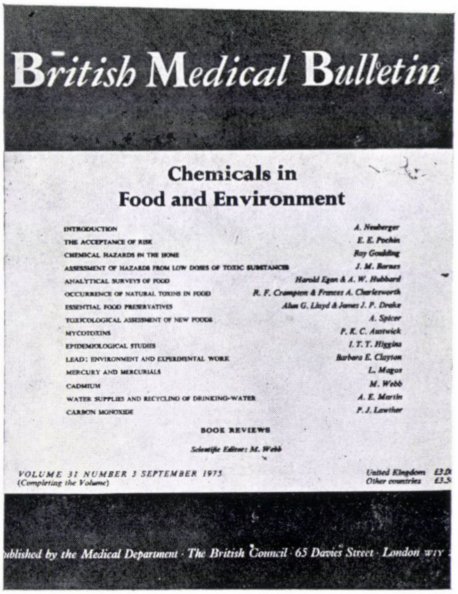
So what the author does is to summarize the basic economic, biological and technical principles of development and present solutions for individual farms. The problem is, here again, that the systems of farms are discussed to varying depths and with different contents and data, so it is difficult to draw conclusions. Collecting and publishing solutions for the different farms is, however, a very useful thing, because it offers a cross-section and provides a basis for more detailed information.

The book is rich and concise in content and presents a wide range of data based on literary sources, the authors' own investigations and reports made by the farms. It is a pity that occasional misprints occur in it.

Hungarian experts will certainly find this book worth reading, and it will provide useful information for foreign experts too, since it contains good ideas for research and development work and for the formulation of directives for management.

M. Tóth

British Medical Bulletin, 1975, 31, 3.



British Medical Bulletin

Chemicals in
Food and Environment

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BOOK REVIEWS

Scientific Editor: M. Webb

VOLUME 31 NUMBER 3 SEPTEMBER 1975
(Completing the Volume)

United Kingdom £3.0
Other countries £3.5

ublished by the Medical Department · The British Council · 65 Davies Street · London W1Y

It has always been the practice of the British Medical Bulletin, published by the Medical Section of the British Council, to present in each of its yearly three numbers analytical and summarizing articles on a subject of current interest, written by the best known experts on the subject. These valuable summaries have enabled the Bulletin to make a great contribution to the solution of medical problems both at home and abroad.

No. 3 of Vol. 31 provides a survey on the subject of "Chemicals in foods and in the environment" in the form of analytical articles.

The rapid industrial development of the past decades has resulted in revolutionary changes in the most diversified fields of life. However, together with the positive changes, the living world, including the environment of man, has become considerably threatened. This process is still continuing, and the more efficient protection of the living world from environmental damage has become a daily necessity in all fields of life. Every attempt to solve this undoubtedly complicated and manifold problem with scientific thoroughness is therefore to be welcomed.

Of all the types of pollution special emphasis should be laid on chemicals, since these cause the greatest damage to the environment.

Today industry and agriculture are becoming more and more closely allied. The utilization of chemicals by agriculture has multiplied all over the world during the last few decades, thereby increasing the danger to the environment.

Acquiring a thorough knowledge of the harmful chemical contents of foodstuffs and of the environment is undoubtedly a complex undertaking, so a presentation of the major results attained in this field by medical science, together with the correlations revealed will certainly be very useful to agricultural experts. The publications found in Vol. 31 No. 3 of the British Medical Bulletin and reviewed briefly below serve this purpose.

Pochin, E. E.: The acceptance of risk. The author discusses the extent of risk involved in various occupations, where considerable differences are shown in the trend of accidents and the frequency of occupational diseases. It is worth noting that in the United States the number of accidents occurring in agriculture is relatively high, probably due to the high level of mechanization and chemization. One deficiency of the paper is the absence of an objective evaluation of the damage caused by smoking, as a special case of accepting risk.

Goulding, R.: Chemical hazards in the home. Chemical sources of danger occurring in the home are detergents, medicines, horticultural chemicals, toadstools, alcohol, etc. These sources of danger can be eliminated most efficiently either by the removal, or if necessary, by the careful isolation of poisonous materials in the home, combined with an extensive information campaign.

Barnes, J. M.: Assessing hazards from prolonged and repeated exposure to low doses of toxic substances. The practically unobserved effects of low doses of particularly powerful poisons insidiously attack and destroy living organisms. These harmful substances are very difficult to trace, and new ones are constantly being identified. The analogy with the vitamins is very interesting, as are the repeated and convincing reports of the damage caused to the nervous system by certain insecticides (DDT, etc.) which are able to accumulate. Malignant tumours, severe lesions caused by traces of chemical carcinogens (e.g. dimethylnitroseamine), belong to this range of subjects. The detection of traces of toxic substances in the environment and in foodstuffs requires particularly sensitive analytical methods.

Egan, H. — Hubbard, A. W.: Analytical surveys of food. In this paper the authors touch upon the question of the food control system introduced in the United Kingdom, and demonstrate by means of examples (e.g. the detection of mercury compounds in fish products) its importance for public health. Special attention is paid in the paper to the strategy of food contamination analysis. The detailed information given in the paper on trace contaminants in food (e.g. pesticides, carcinogens) is highly valuable.

Crampton, R. F. — Charlesworth, F. A.: Occurrence of natural toxins in food. It is not only from external sources that toxic substances may be introduced into foodstuffs. The original raw material of the food itself may contain known or as yet unknown toxins. For example, a great number of natural carcinogens have been demonstrated in foodstuffs, and these are clearly summarised in the paper. The oxalates as food toxins are dealt with in detail. The detection of natural toxins found in foodstuffs may promote the diagnosis of many mysterious diseases. The absence in the paper of references to toadstools is to be regretted.

Lloyd, Alun G. — Drake, James J. P.: Problems posed by essential food preservatives. One of the greatest problems facing the food industry is the preservation of foods. The most important storage method is conservation with chemicals. Some of the chemicals used for preservation have been found to be harmful to health. The endeavour to use less harmful chemicals — as summarised in the paper — is therefore readily understood. The abundant literary material gives thorough information on the subject.

Spicer, A.: Toxicological assessment of new foods. It is natural, from what has gone before, that new foodstuffs must be subjected to a systematic toxicological analysis prior to being put on the market. In these analyses both the natural toxins and those introduced from outside should be taken into consideration. We agree with the author that special attention should be paid to the sanitary aspects of utilizing new proteins. The complete removal of chemical substances harmful to the human organism from food is almost impossible, therefore the relatively safe norms established for known toxins should be extended to further chemicals.

Austwick, P.K.C.: Mycotoxins. Apart from Claviceps, the fungus which produces the classical mycotoxins, a large number of other mycotoxins are known today, which are of a different nature than the ergot alkaloids. The paper gives a clear survey of the mycotoxins known so far. Mycotoxin producing tumours in animals are summed up in a separate table. There is an urgent need to study the correlation between the mycotoxins and many unknown diseases.

Higgins, I. T. T.: Importance of epidemiological studies relating to hazards of food and environment. The author gives examples to illustrate the role of epidemiology in detecting diseases induced by chemical substances. Particularly detailed information is given in the paper on the environmental causes of cancer. Much more information is needed about the potentially health-destroying substances occurring in the environment of man; however, this requires the improvement of the methods of analysis, particularly that of methods ensuring large-scale routine analysis.

Clayton, Barbara E.: Lead: the relation of environment and experimental work. The harmful effect of lead on health has long been known, but modern medical science has revealed a further damaging feature. Lead has a particularly adverse effect on the activity of vital enzymes. The relationship between lead and intellectual development is dealt with in a separate chapter. Tap-water, soil and air are important sources of lead contamination. The reduction, both in the environment and in foodstuffs, of contaminations by lead, an element employed in many spheres, is an urgent task.

Lagos, L.: Mercury and mercurials. Salient particulars are published by the author on the conditions of biotransformation and on concentration observed in ecosystems. A separate report is given on inorganic and organic mercurial substances and on the metal mercury itself. A critical review of short carbon chain alkyl mercurial compounds, which have lately been in the centre of interest, is also presented.

Webb, M.: Cadmium. The author presents a comprehensive study on cadmium, a poison known to act in a particularly insidious way. The occurence and excretion of cadmium are especially interesting. The interaction between cadmium and other fundamentally important metal ions and the adverse effect of cadmium on the functions of these ions are crucial points.

Martin, E. A.: Water supplies of the future and the recycling of drinking-water. There is a shortage of harmless drinking-water all over the world. The author surveys the available sources of water with special regard to the future. Water pollution and various up-to-date methods of water purification are discussed. Information about microbial sources of danger is given in a separate chapter, but perhaps some mention should have been made of the damaging substances present in algae, which are as yet largely unknown.

Lawther, P. J.: Carbon monoxide. A clear description is given of carbon monoxide, one of the longest-known gases harmful to health.

Although there is nothing basically new to be found in the compilation, the evaluation of the damaging effects of carbon monoxide may nevertheless be very useful to those engaged in environmental protection.

To sum up, in Vol. 31 No. 3 of the British Medical Bulletin abundant literary data are presented on harmful chemical substances in foodstuffs and in the environment. It may be of great use to both physicians and agricultural experts working on this subject.

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INDEX

VARIA

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FORUM

CONTRIBUTIONS

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A kézirat nyomdába érkezett: 1976. IV. 21. — Terjedelem: 23,75 (A/5) ív 86 ábra

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